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14. ABSTRACT
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(a) Papers published in peer-reviewed journals (N/A for none)

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Special Publication 13

Frontiers of Karst Research

Proceedings and recommendations of the workshop held
May 3 through 5, 2007 in San Antonio, Texas, USA

2008

Edited by
Jonathan B. Martin
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KARST WATERS INSTITUTE

Special Publication 13

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Cover Photo: Cylindrical stalagmites are rapidly becoming an important paleoclimate archive. This display of these speleothems is from Virgin Cave, Guadalupe Mountains, New Mexico. Photo by David Bunnell/Under Earth Images.

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PREFACE

Some of the world's most exotic landscapes and rare and endangered organisms are in regions characterized as karst, a term used to describe landscapes containing the caves, sinkholes, sinking streams and springs that form in soluble rock, especially limestone. Until fairly recently research on karst in the U.S. was carried out in a relatively few universities by a few individuals and their students. Research was tightly focused on the details of cave ecosystems, karst processes and karst hydrogeology. Also until fairly recently, the karst lands of the United States were located in rural areas where their special characteristics caused relatively few problems. Both situations have changed. Research on karst is now underway in many institutions, some with substantial groups of faculty and students specializing in the subject. Urbanization and population growth have pushed into the karst lands so that water supplies, ground water contamination, and land hazards such as sinkhole collapse and sinkhole flooding create increasingly large economic and human impacts. Karst research has been found to have much wider scientific and societal implications than previously recognized (e.g. paleoclimate archives and water resources). For the biologists and microbiologists, caves have been found to be good models for extreme environments, a subject of a great deal of current interest.

The broad array of intellectual challenges facing karst researchers can best be met by multidisciplinary teams who focus their efforts on understanding related problems that cross traditional disciplinary boundaries. Such efforts will require extensive planning and highly focused cooperation among disparate groups of researchers. The workshop reported in this document had, as its objective, bringing together a representative cross-section of these scholars so that the current state of knowledge could be assessed and some guidance constructed for the future.

The Karst Waters Institute's workshop on "Future Directions in Karst Research" took place in San Antonio, Texas, between May 3 and May 6, 2007. Attendees included 86 scientists from across North America and Europe. An attempt was made to include all aspects of karst science including biology, biogeochemistry, microbial ecology, hydrogeology and geomorphology.

The workshop was organized into a series of alternating plenary and breakout sessions. The workshop was initiated with a plenary session in which the leaders of each breakout group presented their views on the current state of knowledge in their discipline. For organizational purposes, the breakout groups were broadly divided into topics designated as Hydrologic Modeling; Geochemistry and Climate; Ecosystem Function; Biodiversity; Biological Evolution; Geomicrobiology; and Applied Issues, although these titles and topics evolved over the course of the workshop.

Following the initial plenary session, participants divided themselves into seven breakout or focus groups. These initial breakout sessions lasted through the end of the first day and continued through the morning of the second day. In the early afternoon of the second day, a second plenary session was convened during which focus group leaders presented the results of disciplinary discussions within their group. During this session, the workshop participants as a whole began to discuss research needs that cut across disciplines. The remainder of the second day was spent in breakout sessions during which the research needs of individual disciplines were refined to reflect the input from the general discussion. The workshop ended on the morning of the third day with a final plenary session, where there was an open discussion of the findings of the meeting, the critical research questions that had been identified at the workshop, and how the science should best move forward.

Each focus group leader selected a few experts to participate in their group's discussion. In addition, general participants could select the focus group closest to their interests and were free to move between focus groups. Two cross-disciplinary reporters were assigned to rove between the groups, transferring ideas and suggestions.

The workshop report consists of three parts. In extensive discussions following the formal close of the workshop, the organizers and the focus group leaders attempted to identify key areas with the greatest potential for advancement and to distill specific suggestions for future research. These distillations appear in Part 1 – Opportunities and Recommendations. The focus group leaders were asked to prepare written summaries of their plenary presentations. These appear as signed reports in Part 2. Focus group leaders also prepared summaries of the discussions within their group which were reviewed and revised by Focus Group members. These appear in Part 3.

The workshop organizers were immensely pleased at the number of scientists who took the time and expense to attend. The large attendance gave the workshop a broad base and an increased likelihood that the report does indeed represent a cross-section of the scientific community. To all participants, we say Thank You.

Part 1

FRONTIERS OF KARST RESEARCH OPPORTUNITIES AND RECOMMENDATIONS: THE NEXT FRONTIER

FRONTIERS OF KARST RESEARCH OPPORTUNITIES AND RECOMMENDATIONS: THE NEXT FRONTIER

Karst is the term applied to terrains underlain by soluble rock: mainly limestone, dolomite and gypsum. Karst is a terrain characterized by caves, sinkholes, mobile soils, sinking streams, fast-flow underground drainage systems, large springs, and an assortment of weirdly shaped landforms sculptured on the bedrock. A recent investigation estimates that karst makes up 12.5% of the Earth's land surface.

Karst impacts human-kind in many ways. Because of the efficient movement of water into the subsurface, surface streams are frequently dry or non-existent. Water wells drilled in karst may produce abundant yields or none at all. Karst springs are widely used as water supplies but are at risk of contamination. Soils are easily flushed into the subsurface resulting in "karst desertification." Soil instability can allow foundations to be undermined resulting in extensive property damage. Sinkhole collapses are a continuous land-use hazard.

Caves, of many sizes and patterns, are the hallmark features of karst. Caves have been of interest to humans for millennia as dwellings, refuges, and places of worship. Only in the past several centuries have caves become significant objects of scientific investigation. Caves were found to contain such a fascinating assortment of processes, minerals, unique organisms, and sedimentary and paleontological deposits of various kinds that the early investigators invented a new science: speleology. Speleology was inwardly looking. Scientific disciplines from outside – biology, geology, hydrology, mineralogy, paleontology, archaeology – were all focused on understanding what was going on in the caves.

Greatly accelerated research on caves and karst over the past several decades suggests that the speleologists got it backward. The value of cave and karst related research is not in any pressing need to understand karst regions as such but for the insights that karst regions provide to all of the contributing sciences. It was to identify these promising research opportunities that the Workshop on Frontiers of Karst Science was convened in San Antonio, Texas in early May, 2007. A series of focus group sessions identified subjects for future research that have the potential for important advances that extend far beyond the research sites themselves. These recommendations are summarized in the sections that follow. Disciplinary state-of-the-art keynote presentations are given in Part II. The full reports of the Focus Groups are given in Part III.

This summary of research opportunities is organized by scientific context. It does not follow exactly the Focus Group reports because of overlap and inter-relationships of the subjects discussed by the Focus Groups.

AQUIFERS AND GROUND WATER IN CARBONATE TERRAINS

Opportunities

A reasonable understanding of the components and functioning of karst aquifers has been established and important progress has been made on quantitative modeling of aquifer behavior. As is frequently the case, present understanding reveals a new layer of scientific questions that would be valuable to investigate.

a) Channel hydraulics: There has been some success at modeling flow in conduits in terms of pipes. However, aquifers may contain reaches of open channel flow interspersed with reaches of pipe flow. Further, the proportions of each may vary with flow volume, flood flows filling more of the system than base flows. The quantification of this problem in fluid hydraulics would be an important step forward.

b) Turbulent flow: Although difficult to parameterize, turbulent flow processes may explain many observations in karst flow and transport such as dispersion and tailings in tracer breakthrough curves. Computational fluid dynamics may produce a quantitative model at the laboratory scale but calculations at the catchment scale are a more difficult problem.

c) Unsaturated flow: Most models and calculations have focused on aquifer behavior in the phreatic zone. Complete models must include the role of the epikarst as a temporary storage and retardation factor and must also include unsaturated flow in the vadose zone. This would include film flow in wide fractures and shafts as well as saturated storm flow in smaller fractures.

d) Chemical evolution: What are the mechanisms that control the chemical evolution of water along a flow path from surface water to ground water and return to the surface? This is one of the best developed aspects of karst but much detail remains to be determined. For example, the role of biofilms and microbial activity in the dissolution and precipitation processes.

e) High resolution chemical measurements: High resolution temporal and spatial analysis of geochemical and physical variability, particularly for transient events such as storm flows, will be extremely valuable for the understanding and modeling of the system. Numerous studies have demonstrated that rainfall, changes in water level, spring discharge, turbidity, and concentrations of natural and anthropogenic chemical constituents may occur on a timescale of hours.

f) Sediment transport: Well-developed karstic aquifers carry a load of clastic sediments which may be stored in the conduits at base flow and flushed during storm flow. Sediments contribute to the transport of contaminants. The mechanism of sediment transport and thresholds for their movement need to be quantified.

g) Variable density flow: Flow in island and coastal karst is modified by the variability in salt concentration. A model for the flow is needed that takes account of advective flow and possible density-driven convection.

h) Multiphase flow: Primarily, multiphase flow calculations would apply to contaminant transport, particularly massive spills of non-aqueous phase liquids. However, similar approaches might be useful for air/water interfaces. The fluid dynamics of such flows in karst aquifers is a challenging problem.

Constraints and Needed Resources

a) Instrumented watersheds: Calculations and model-building are only as good as their agreement with observation. A highly instrumented watershed that would provide detailed data on recharge, flow, and discharge over a reasonably long time scale would provide a base against which models could be tested.

b) Dispersed recharge: Methods need to be developed, including hydrological, geophysical, and remote sensing techniques for measuring recharge through the soil zone and the underlying epikarst. Quantitative data on flow and storage through dry, wet, and storm periods are also needed.

c) Characterization of the conduit system: Although the conduit system dominates the flow pattern, it is extremely difficult to detect by other than direct exploration. Conduits comprise a small volume fraction of the aquifer and traditional drilling and geophysical techniques are of limited usefulness. There is need for novel geophysical approaches that would reveal portions of the conduit system that cannot be reached by explorers.

Limitations and Concerns

The primary limitation is one of commitment. Many previous hydrologic studies of karst aquifers have been limited to relatively short term observations, rarely more than one water year, often much less. To make progress, detailed measurements need to follow the behavior of benchmark aquifers over many water years.

KARST AS HUMAN HABITAT

Opportunities

Living on karst is subject to problems broadly categorized into water supply issues and land use and land management issues. These issues are, in effect, the applied side of karst hydrogeology. Aspects in most urgent need of attentions are:

a) Land use impacts: What are the impacts of land use changes and climate change on water resources in karst areas? Can the sources of impact be separated? Such impacts present a great opportunity for long range quantitative monitoring of discharge and water quality.

b) Contaminant storage: How and when are contaminants stored and later transported through karst aquifers? This will vary with the type of contaminant, the details of aquifer permeability, and the frequency and intensity of storm flows.

c) Sediment and contaminant transport: What are the relationships between sediment transport and contaminant transport? The relations between turbidity curves, contaminant chemographs, and hydrographs of karst springs and cave streams in response to storm flows of various magnitudes would contribute a great deal to this question.

d) Aquifer yields: Determining sustainability and specific yield of karst aquifers is, if possible at all, difficult and expensive. Development of new methods would greatly enhance resource allocation and water supply planning in karst regions.

e) Sinkholes and land instability: Although there are hundreds of case studies documented, there are few fundamental relationships between soil properties, drainage basin characteristics and the risk of sinkhole collapse. Sinkhole theory of sufficient accuracy to be an effective prediction tool would be of immense value to land managers.

Constraints and Needed Resources

Most of the specific research opportunities require long term observations. To accumulate the necessary data a network of dedicated research field sites could be instrumented and managed for systematic data collection.

The study of contaminants in karst aquifers is difficult because of the very rapid transport of both water and contaminants. Because serious contaminant spills cannot be introduced under controlled conditions, an alternative is a “Red Team” approach in which a selected group of researchers stand ready to mobilize within hours when a serious spill occurs. This approach would allow data collection on the leading-edge of contaminant behavior which is otherwise rarely observed.

Limitations and Concerns

Although numerous potential dedicated field sites exist, continuous data collection requires up-to-date instrumentation and staffing to assure that the records are complete and collected with the appropriate temporal and spatial resolution.

There are often legal and access barriers to conducting research on contaminated sites due to regulatory issues and liability concerns by land owners. Formal arrangements would be needed in advance if the “Red Team” approach is to work.

CAVES AS CLIMATE ARCHIVES

Opportunities

Cave sediments, both clastic deposits of clays, sands and gravels and speleothems (chemical cave deposits – mostly calcium carbonate) carry imprints of surface conditions at the time of their deposition. Combined with techniques for obtaining high accuracy, high temporal resolution dates for these deposits, cave sediments are rapidly becoming one of the most important sources of paleoclimate information on time scales that range from the historic to the Pleistocene.

a) Clastic sediments as climate records: Many caves contain deposits of clastic material that can be dated by cosmogenic isotope methods. Such dates provide a chronology for the development of the cave passages and thus the nearby surface topography. Combining accurate dates with a consideration of the transport mechanisms for the sediments, would give insight into the hydrologic conditions in existence at the time of deposition. Clastic sediment dates have a low time resolution but a much longer time scale – extending into the Pliocene and record rare but major climatic events.

b) Relationship between climate change and water availability: Calcite growth in speleothems is very dependent on drip rate from cave ceilings and thus on rainfall. Studies to date have been very promising. Given the large spatial and temporal distribution of speleothems, further studies can provide unprecedented insight to:

- The underlying mechanisms that have modulated past climate variability across multiple time scales from seasonal to millennial.
- The frequency and magnitude of extreme events such as droughts, floods, and cyclones.
- The implications of past variability on future climate change.

c) Climate records in terrestrial environments: Paleoclimate reconstructions have relied heavily of marine and ice core records. Caves are widely distributed in the continental interiors. Profiles of oxygen and carbon isotopes, color and luminescence banding, and trace element profiles provide a wealth of climatic proxies. High resolution U/Th dating provides the time scale. The studies made thus far have been highly productive but each cave provides only one spatial point. Much more work is needed to reveal regional and continental scale patterns.

d) Karst and the global carbon cycle: To what extent are karst systems a source or sink for CO_2? The transport of carbon out of soils and into the karst environment and the loss of CO_2 from cave atmospheres and karst waters have not been documented on a landscape scale. Karst represents a loop in the carbon cycle that should be addressed.

Constraints and Needed Resources

a) Speleomap: At present, speleothem studies are individual efforts on the part of university faculty and their students. Access to the data is only through the published literature which often does not include the raw data. Significant resources would be required to build a master data base from which a continental SPELEOMAP could be produced analogous to the marine and lake records used to produce the CLIMAP data set.

b) Calibration: Although speleothems produce outstanding high-resolution climate proxies, it is much less certain how to connect isotope ratios, trace element concentrations, and other time-calibrated data in the speleothem to actual climatic variables on the land surface above the cave. Investigations of the changes in isotope ratios and other parameters along the flow path between the rainfall and the speleothem drip water are absolutely crucial to the interpretation of all other data.

c) Dating: The great value of speleothem records lies in the ability to determine accurate and absolute dates from milligram samples of the speleothem layers. Only a few laboratories have the mass spectrometers and know-how to prepare accurate dates. At present access to dating facilities is through collegial arrangements. Some dating facilities should be supported so that with more formal arrangements they would be open to the entire community.

Limitations and Concerns

Paleoclimate investigations of speleothems have the serious drawback that they are destructive. Typically, entire stalagmites are collected from caves of interest, sectioned, and sampled along the central core. Speleothems are considered to be a valuable resource and caves have high esthetic value. Strong conservation measures should be respected so that collection is minimized and the amount of data obtained is maximized.

a) Improved sampling techniques: Microcoring techniques should be developed that would permit a slender core to be extracted along the growth axis of the stalagmite or other speleothem. It should not be necessary to break off and remove the entire speleothem.

b) Archives: Kilogram quantities of speleothem are removed to provide milligrams of samples. The unused portions of speleothems, along with any characterization data collected concerning them, should be placed in an archive where the material would be available to other investigators without further damage to the caves.

CAVE MICROBIOLOGY

Opportunities

a) Microbial diversity. All three domains of life, Eukarya, Bacteria, and Archaea, and also viruses occur as microscopic life in caves. There are vast gaps in our knowledge of the diversity and distribution of these organisms that needs to be filled. Both geographical variation in cave location and also the substrate – the character of the mineral surfaces – are important.

b) Origins: What is the source of microbes in caves and karst?

c) Adaptation: Do microbes adapt to the subsurface environment?

d) Habitat: Does the subsurface habitat influence microbial community composition?

e) Ecosystem function: How are microbes central to ecosystem function in different types of karst? Are nutrients limiting for microorganisms in the subsurface? Are microbial community structure and function linked?

f) Disturbance: Are microorganisms agents or victims of disturbance?

Constraints and Needed Resources

a) **Better tools**: Better methods and tools for understanding the presence or absence of microbes are badly needed. These should be more inclusive, *in situ* and non-invasive. Instrumentation should also be durable, stable, and economical.

b) Standard practices: The application of traditional microbiological and molecular methods may not be appropriate for cave and karst investigations. Cultivation studies are needed to explore the physiology of indigenous organisms so that better cultivation methods can be developed.

Limitations and Concerns

The limitations are only the usual ones of student interest and availability and necessary facilities for research.

CAVES AND KARST AS HABITATS

Opportunities

Caves act as habitat for limited populations of a limited set of organisms thus making caves useful for the study of ecosystem function and species diversity.

a) What limits productivity in karst? Microbes are key mediators of energy flux in food webs that are exclusively heterographic as well as those fueled by chemoautotrophy. Exploration of factors limiting microbial productivity would be of value. The role of inorganic nutrients in regulating productivity in chemoautotrophic system should be an important avenue of research.

b) Spatial and temporal variation in ecosystem function: Future research should focus on quantifying the spatial and temporal patterns in the delivery of energy and the factors that control these patterns. In particular, identifying the presence of "hotspots" and "hot times" for ecosystem function and the factors that lead to these situations should be addressed.

c) The problem of cryptic species: Cryptic species (genetically distinct, but morphologically identical) are numerous in caves. The mismatch between genetic convergence and morphological convergence is not understood. What is needed is the simultaneous mapping of genetic and morphological diversity on a geographic scale.

d) Mapping Subterranean Biodiversity: Current data on subterranean biodiversity is limited by variations in sampling intensity and frequency, by sampling bias, and by sampling incompleteness. A project is proposed that would sample 250 caves in North America and 250 caves in Europe using standardized sampling protocols.

Constraints and Needed Resources

A needed resource is a selection of karst drainage basins which can be used for long term study. These need to be controlled and instrumented so that observations can be extended over long time periods. Basins (or portions of basins) where the land surface can be manipulated by such means as changing vegetation, soils and water fluxes would be highly desirable.

Study of subterranean biodiversity requires the continuing identification of new species. The basic but unglamorous task is limited by the number of trained taxonomists specializing in the various cave organisms.

Limitations and Concerns

A complete understanding of ecosystem function requires understanding of both mass and energy fluxes through the entire karst system. To obtain this understanding requires communication and cooperation between hydrogeologists, geochemists, and biologists who, in an ideal arrangement, are studying the same system.

The limited number (and aging) of taxonomists may require incentives for new researchers to enter the field and also perhaps some change of orientation in their training – training students to be subterranean specialists rather than group specialists, for example.

CAVES AS EVOLUTIONARY LABORATORIES

Opportunities

Cave animals are emerging as strong model systems for understanding the tempo and mode of evolution. Advances in molecular and genomic techniques now make it possible to use these organisms to understand general questions of evolutionary biology

a) How can we understand the relationship between convergence and divergence, and the interplay between morphology and genetics leading to these two paths? While convergence among disparate taxa is a widespread evolutionary phenomenon, the interactions between convergent and divergent phenotypes and genotypes and how these interactions affect morphologies have not been comprehensively investigated. Focusing research on particularly obvious convergent morphological traits (e.g. depigmentation) and the evolution of associated homologous genes across taxonomies will help our understanding of general trait evolution, as well as the connection between sequence evolution and protein structural constraints.

b) To what extent is the evolution of the cave form controlled by the nature of the karst environment versus intrinsic evolutionary mechanisms? Karst settings offer repeated environments where these types of questions can be investigated. The subterranean habitat exerts strong evolutionary pressures on inhabitants, as evidenced by the particular suite of traits characterized by highly cave-adapted species. Cave-adapted animals, particularly those with close surface relatives, offer the potential for genome-wide comparisons to determine how much of the cave form is genetically hard-wired versus environmentally driven.

c) What is the timescale of evolutionary change? Caves preserve information that is available in few surface habitats, where weathering processes remove the record of past events. Well studied karst systems allow for true hypothesis testing, where hypotheses about subterranean colonization, lineage diversification, speciation, and trait evolution can be generated based on the geology of the system and then tested in the evolution of the species contained within that system.

d) Understanding mutation rates: Mutation rates are essential both to understanding both the divergence times of species and the evolution of forms. Cave species offer interesting systems in which it may be possible to address these questions.

Constraints and Needed Resources

There is need to establish at least two pairs of surface and cave-adapted populations or species as model organisms.

There is need for detailed cooperation between investigators of evolutionary biology and the hydrogeologists and geochemists concerned with the physical evolution of cave systems, their relationship to surface topography, and the isotopic age dating of cave sediments and speleothems.

Limitations and Concerns

These problems are ripe for investigation.

CROSS-DISCIPLINARY OPPORTUNITIES

An immediately obvious impression obtained from wondering between the Focus Groups was the extent of overlap and mutual dependence between the very diverse scientific areas represented. In a certain sense, everyone needs to draw on the concepts and data of everyone else.

Cross-Disciplinary Research

a) **Redox sensitive elements**: What is the fate and transport of redox-sensitive elements and their microbial consequences and feedbacks? Understanding the geochemistry of karst waters requires understanding variable valence (redox) elements such as iron, manganese, and many trace metals. Such understanding requires also understanding the role of microorganisms in catalyzing and mediating reactions. Cooperative research between geochemists and microbiologists is essential.

b) **Integration of geochemical data with hydrological modeling**: Modeling geochemical processes in conjunction with fluid flow is a frontier in hydrologic modeling and would be particularly useful when considering karst dynamics.

c) **Epikarst and the vadose zone**: Investigation of travel times and flow paths through the epikarst and the vadose zone is important to hydrogeology, to contaminant transport, to paleoclimate records in caves, and to ecosystem biology.

d) **Cave development and dating of cave deposits**: The time sequence of cave passage development provides a benchmark for evolutionary biology. Absolute dates on cave deposits, both clastic sediments and speleothems, are critical to paleoclimate studies but are also important benchmarks for other investigations.

Cross-Disciplinary Communication

a) **Direct Communication**: Existing meetings and journals seem to work reasonably well. Better efforts need to be made to develop common language and understanding so that understanding is not masked by jargon. This is particularly important at the international level.

b) **Education**: The "graying" of the karst community is a significant concern. New educational initiatives and enhancement of existing cave and karst programs are needed.

Data Bases

Raw data concerning chemical composition of waters and cave deposits, age dates on speleothems, hydrographs and chemographs of karst springs, species identifications and distributions, and related data are often of value to investigators other than the ones who collected the data. Primary data are rarely published. A central data base with clear-cut rules for access and proper use would be invaluable.

Of greater concern are data bases for the caves themselves. The primary data for caves, their locations, descriptions, and maps, are collected by private groups of cave explorers. The data are held in highly proprietary data bases and there is usually a great deal of concern that the data will be publically released. A productive working relationship between the karst researchers and the cavers is essential.

Archives

Much of the potential research requires collecting samples from caves. This is especially true for paleoclimate studies where entire stalagmites are collected, sectioned, and sampled for dating and isotope analyses. Speleothems are a limited resource and there are strong ethical and conservation reasons for doing as little damage to caves as possible. An archive or repository for cave material, including any previously collected data on chemical, isotopic or petrologic character would allow collected material to be reused, thus minimizing the need for further collections.

The archiving of biological and microbiological specimens should also be considered.

Monitoring Sites, Field Stations, and Field Laboratories

Responses in a karst system can be long term and short term. Long-term (years to centuries) and short-term (hours to weeks) time-series data are useful for understanding the varied response. Such data can only be acquired if funding priority is given to establishing intensive monitoring networks in karst systems. NSF's proposed Hydrologic Observatories would help achieve this goal. Needed in parallel would be monitoring networks that cover a maximum of aquifer behavior and climatic settings.

Part 2

TODAY'S FRONTIERS

TODAY'S FRONTIERS

It is difficult to plan for the future without knowing where you are at the present. All aspects of karst science have made great strides in the past few decades. It is from this wealth of new knowledge that the workshop participants were able to estimate the knowledge needs of the future.

Each Focus Group leader was asked to prepare a short report on today's state of knowledge concerning karst in the subject area of the Focus Group. These were not intended to be comprehensive reviews but rather a summary of the highlights. These reports were presented to the entire workshop and were intended to act as a launch point for discussion. The Focus Group leaders also prepared written versions of their remarks. These are reproduced in the pages that follow.

These summary papers contain extensive bibliographies which should guide the reader to the very extensive primary literature.

Modelling Karst Hydrodynamics

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[This article is a lightly edited version the article of the same title that appeared as Chapter 10 in *Methods in Karst Hydrology*, Nico Goldscheider and David Drew, Eds., Taylor and Francis, London, p. 201-222 (2007). It is reproduced here with the kind permission of Taylor and Francis, Publishers.]

INTRODUCTION

Mathematical models are exact tools for the quantitative representation of the hydraulic behaviour of aquifer systems. The reconstruction of a groundwater flow field, which is consistent with a given hydraulic conductivity field and given boundary conditions, nearly always requires the use of numerical models (Király 2002). Analytical models of groundwater flow have been developed since the late 1800s. Numerical groundwater flow models have been applied since the 1960s, and their utilisation for flow through a porous medium has become every-day practice since then. However, the application of numerical methods in karst hydrogeology demands a specially adapted modelling methodology (Palmer *et al.* 1999). This is because of particular complexities associated with the large heterogeneity of a flow field (Király 1994).

The first step in any modelling study is the schematic representation of a real system. A conceptual model consists of the applied differential equations, aquifer geometry, and of a set of flow parameters, boundary conditions and initial conditions. The hydraulic parameter fields applied in groundwater flow models are usually obtained with an interpolation between discrete observations. Because of the large heterogeneity and high contrast in hydraulic conductivity, interpolation techniques cannot be employed for the characterization of karst aquifers. The most demanding task in karst modelling is the definition of continuous hydraulic parameter fields.

The second problem is the selection and the development of an appropriate computer code based on numerical methods, which allows the equations defined in the abstract scheme to be solved. Large heterogeneities require the introduction of special features into the numerical models, such as the combination of discrete 1-D elements with a three-dimensional continuum or the double continuum representation of the flow medium.

The third problem is related to the transfer of the simulated results to a real system. Simplifying assumptions made in the conceptual and numerical models must appear as uncertainties in the simulation results. Because of the high degree of heterogeneity, these uncertainties are much larger in karst systems than in most porous unconsolidated aquifers.

This state-of-the-art report aims to provide a brief overview on karst modelling techniques and related problems. It is intended to assist the modelling hydrogeologist in the selection of appropriate tools as well as the definition of suitable parameterisation techniques.

CONCEPTUAL MODELS OF KARST SYSTEMS

Every sound conceptual model of karst systems incorporates heterogeneity and accordingly the duality of hydraulic flow processes. These include the duality of infiltration, storage, groundwater flow, and discharge processes. Continuum flow (often termed diffuse flow) processes are active in low permeability matrix blocks or slightly fissured limestone beds, while concentrated flow processes can be observed in a discrete conduit network. Moreover, most conceptual models distinguish three main zones in the vertical direction. These are: soil zone and epikarst, unsaturated or vadose zone, and phreatic zone. Although most conceptual models include similar structural features, the flow and storage processes assigned to them display large variations.

According to the conceptual model of Mangin (1975), the main conduit system transmits infiltration waters towards a karst spring, but is poorly connected to large voids in the adjacent rocks, referred to as the 'annex-to-drain system'. Mangin (1975) associates storage to the 'annex-to-drain system' and also introduced the concept of epikarst, i.e. a shallow, high-permeability karstified zone just below the soil zone. It is believed to act as a temporary storage and distribution system for infiltrating water, similar to a perched aquifer. It is assumed to channel infiltrating water toward enlarged vertical shafts, thereby enhancing concentrated infiltration.

Drogue (1974, 1980) assumed, that the geometric configuration of karst conduit networks follow original rock fracture patterns. Joints constitute a double-fissured porosity system. This network consists of fissured blocks with a size in the order of several hundred metres, separated by high-permeability low storage conduits. Every block is dissected by small scale fissures or fractures with considerably larger storage, with low bulk permeability.

The conceptual model proposed by Király (1975, 2002) and Király *et al.* (1995) combines the conceptual models of Mangin (1975) and Drogue (1980). This model involves the epikarst and a hierarchical organization of the conduit networks. It also comprises the hydraulic effect of nested groups of different discontinuities. According to Király and Morel (1976a), two classes of hydraulic parameters can adequately reflect the hydraulic behaviour of karst systems. Carbonate aquifers can be considered as interactive units of a high-conductivity hierarchically organised karst channel network with a low-permeability fissured rock matrix. While Mangin (1975) associates storage to the 'annex-to-drain system', Drogue (1980) and Király (1975) associate it to the low-permeability matrix. The strength of Király's (1975) conceptual model is based on the fact that it has been tested quantitatively and verified by numerical models (Király and Morel 1976 a, b, Király *et al.* 1995). A schematic representation of this concept is provided by Doerfliger and Zwahlen (1995) in Figure 1.

MODELLING APPROACHES

Two, fundamentally different modeling approaches exist for studying and characterizing karst hydrogeological systems.

Global models (lumped parameter models) imply the mathematical analysis of spring discharge time series (hydrographs) that are believed to reflect the overall (global) hydrogeological response of karst aquifers. According to this approach, karst systems can be considered as transducers that transform input signals (recharge) into output signals (discharge). With the acquisition of spring discharge data being relatively simple, these models have already been employed since the beginning of the last century. Traditional global modelling techniques do

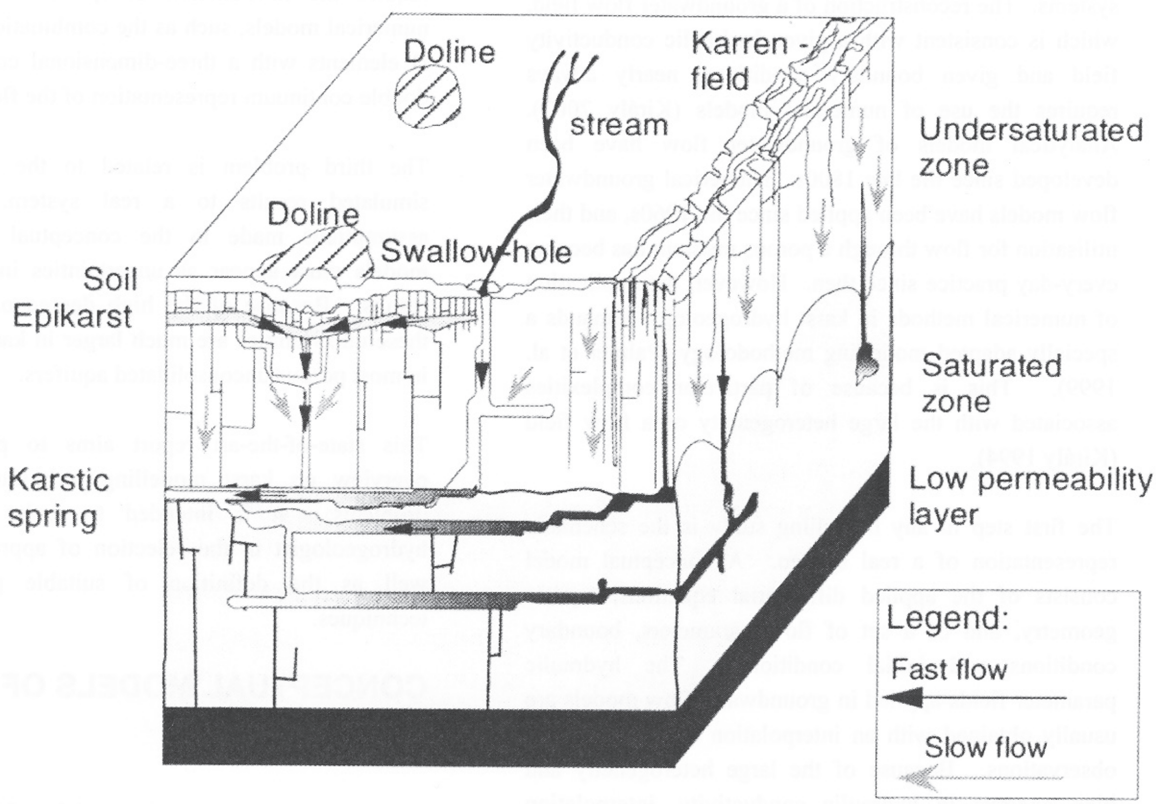

Figure 1: Conceptual model of karst aquifers (Doerfliger and Zwahlen 1995).

not take into account the spatial variations within the aquifer and cannot provide direct information on the aquifer geometry, hydraulic parameter fields nor physical parameters. However, some of these techniques combined with other exploration methods may be used for the estimation of hydraulic parameters and conduit spacing, information required for distributive flow models.

For the quantitative spatial simulation of groundwater flow fields, distributive models are needed. The application of distributed parameter methods requires the subdivision of a model domain into homogeneous sub-units, for which groundwater flow can be described by flow equations derived from basic physical laws. Distributive models can consider both spatial and temporal variations of hydraulic parameters and boundary conditions, and thus require detailed information on aquifer geometry, hydraulic parameter fields, and recharge conditions.

GLOBAL METHODS

The measurements of discharge with time at the outlet of a karst aquifer provides integral information on the hydraulic behavior of the entire system. The following two types of spring hydrograph analytical methods can be distinguished (Jeannin and Sauter [1998]).

Single Event Methods deal with the global hydraulic response of the aquifer to a single storm event. It is widely accepted, that the hydraulic effect of three fundamental factors are reflected in the global response of a karst aquifer: Recharge, storage and transmission (flow). Most of the existing techniques for the analysis of spring hydrographs allow the identification of integral parameters of karst properties in a qualitative but not quantitative sense. However, most of these methods are based on simple, or sometimes more complex, cascades of reservoirs and involve physical phenomena. Furthermore, some of these methods provide semi-quantitative relationships between the pattern of the global hydraulic response and hydraulic parameters as well as some geometric properties of an aquifer. Therefore, it is more appropriate to speak of "grey box models" rather than "black-box models".

Time Series Analyses relate the global hydraulic response of karst systems to a succession of recharge events. Univariate time series analytical methods can identify cyclic variations. Bivariate time series analyses are designed for the analysis of the relationship between input (recharge) and output (discharge) parameters for different karst systems. These methods are based solely on mathematical operations, and the coefficients obtained cannot directly be related to physical phenomena. Time-series analyses provide limited information concerning the physical properties of the system itself. Consequently, they are "black-box models". On the other hand, the interpretation of the results of such models can sometimes be related to some geometric or hydraulic features of karst systems.

Single Event Methods (Grey Box Models)

An infiltration event over a karst terrain results in a hydrograph peak at the karst spring, which is delayed in time relative to the storm event. This peak can generally be subdivided into three main components: rising limb, flood recession and baseflow recession (Figure 2). The latter is the most stable section of any hydrograph, and also the most characteristic feature of the global response of an aquifer. This is because baseflow recession can be assumed as that segment of a hydrograph least influenced by the temporal and spatial variations of infiltration.

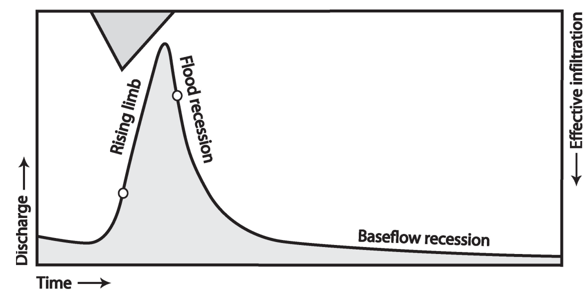

Figure 2: Typical features of a spring hydrograph peak. White dots indicate inflexion points that correspond with maximum infiltration and the end of an infiltration event (Király 1998a, from Kovács 2003).

The mathematical description of baseflow recession provided by Maillet (1905) is based on the depletion of a reservoir, and assumes that spring discharge is a function of the volume of water in storage. This behaviour can be described by the following exponential equation:

$$Q_{(t)} = Q_0 e^{-\alpha t} \quad (1)$$

where Q_t is discharge [L^3T^{-1}] at time *t*, Q_0 is the initial discharge [L^3T^{-1}] at an earlier time, and α is the recession coefficient [T^{-1}] usually expressed in 1/day. Plotting a discharge hydrograph on a semi-logarithmic graph reveals a straight line with the slope -*a* for favourable conditions. This equation is adequate for the description of the baseflow recession of karst systems.

According to Kovács (2003) and Kovács *et al.* (2005), the baseflow recession coefficient can be used for the derivation of important information about aquifer hydraulic parameters and conduit network characteristics. The analytical formulae provided by the above authors are based on a simple conceptual model, which involves a regular network of high permeability conduits coupled hydraulically with a low-permeability matrix (Figure 3). The recession coefficient of a karst spring discharging from such a system reflects the structure and hydraulic parameters of the conduit network and the low permeability matrix.

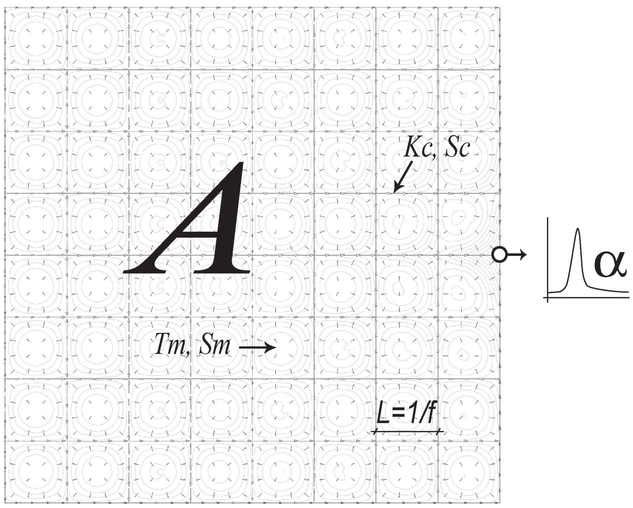

Figure 3: Conceptual model of karst systems according to Kovács (2003) and Kovács *et al*. (2005). Characteristic model parameters are: Transmissivity of the low-permeability matrix (T_m [L^2T^{-1}]), storage coefficient of low-permeability matrix (S_m [L^{-1}]), conductance of karst conduits or fractures (K_c [L^3T^{-1}]), storage coefficient of karst conduits (S_c [L^{-1}]), spatial extent of the aquifer (A [L^2]), and the spatial frequency of karst conduits (f [L^{-1}]).

Numerical studies by the above authors showed that the dependence of the recession coefficient on aquifer properties cannot be described using a single formula, but that it follows two significantly different physical principles depending on the overall configuration of the hydraulic and geometric parameter fields (Figure 4, Figure 5).

The baseflow recession of karst systems is controlled by the hydraulic parameters of the low-permeability matrix, and conduit spacing. This flow condition is referred to as matrix-restrained flow regime (MRFR). The baseflow recession coefficient of karst systems can be expressed as follows:

$$\alpha_b = \frac{2\pi^2 T_m f^2}{S_m} \qquad (2)$$

The baseflow recession of fissured systems and poorly karstified systems is influenced by the hydraulic parameters of fractures/conduits, low-permeability blocks, fracture spacing, and aquifer extent. This flow condition has been defined as conduit-influenced flow regime (CIFR). The baseflow recession of fractured systems can be expressed as follows:

$$\alpha_h \approx \frac{2}{3}\frac{K_c f}{S_m A} \qquad (3)$$

The importance of these hydrograph analytical formulae lies in their potential for providing crucial input parameters necessary for distributive groundwater flow models. They also reveal some fundamental characteristics of the recession process of strongly heterogeneous hydrogeological systems, and make the estimation of aquifer parameters and conduit spacing possible from spring hydrograph data.

Forkasiewitz and Paloc (1967) extended Maillet's approach to the entire recession process by decomposing hydrograph peak recession limbs into three exponential components (Figure 6). The authors assumed that the components reflect three individual reservoirs, representing a conduit network, an intermediate system of well-integrated karstified fractures, and a low permeability network of pores and narrow fissures. However, the analysis of spring hydrographs simulated by numerical models performed by Király and Morel (1976b), and later by Eisenlohr *et al.* (1997a) showed that different exponential hydrograph segments do not necessarily correspond to aquifer volumes with different hydraulic conductivities. Three exponential reservoirs can be fitted to the hydrograph of a karst system consisting of only two classes of hydraulic conductivities. The intermediate exponential could simply be the result of transient phenomena in the vicinity of the high hydraulic conductivity channel network.

Other authors proposed to describe the recession process by employing different mathematical functions. Drogue (1972) described the whole recession process by using one single hyperbolic formula. In contrast, Mangin (1975) distinguished two components on the recession curves, and associated them to the discharge from the unsaturated zone and the saturated zone, respectively.

Time Series Analysis

Time series analyses imply the mathematical analysis of the response of karst systems to a succession of rainfall events. Because these methods are solely based on mathematical op-

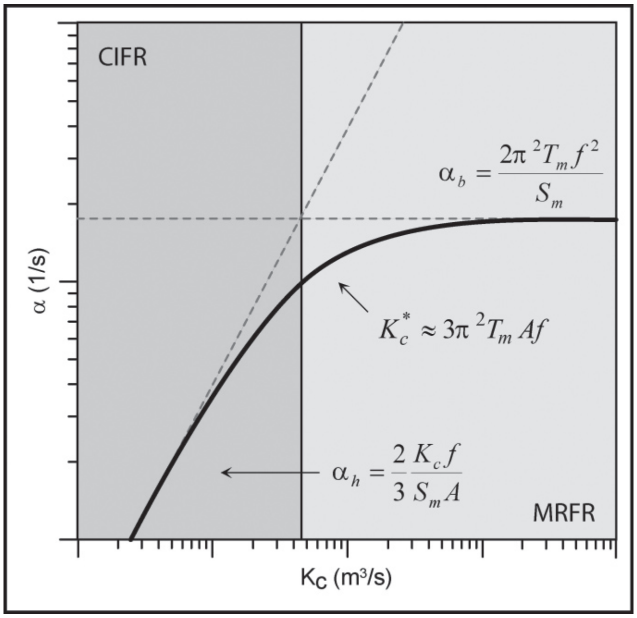

Figure 4: Graphical representation of the relationship between recession coefficient and conduit conductance on log-log graph. Kc* represents the threshold value of conduit conductance. Higher values entail matrix-restrained baseflow (MRFR), while lower values result in conduit-influenced baseflow (CIFR). Modified after Kovács (2003) and Kovács *et al.* (2005)

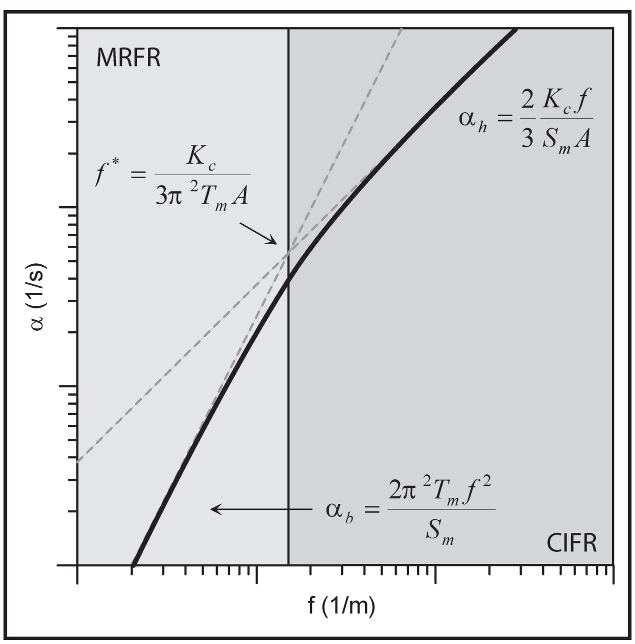

Figure 5: Graphical representation of the relationship between recession coefficient and conduit frequency on log-log graph. f* represents the threshold value of conduit frequency. Higher values entail conduit-influenced baseflow (CIFR), while lower values result in matrix-restrained baseflow (MRFR). Modified after Kovács (2003) and Kovács *et al.* (2005).

erations, they fail to provide information on physical functioning of karst systems, and can be used mainly for prediction and data compilation purposes. Most of the methods used in time series analysis were principally developed by Jenkins and Watts (1968), and were applied to karst systems by Mangin (1971, 1975, 1981, 1984). Detailed explanation of time-series analytical techniques is provided by Jeannin and Sauter (1998).

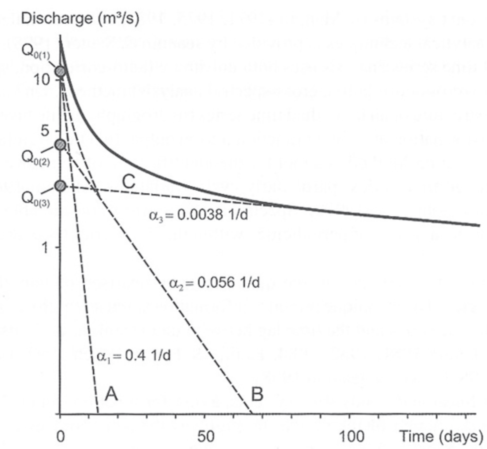

Figure 6: Decomposition of recession curves according to Forkasiewicz and Paloc (1967).

Conventional time series analysis uses both univariate (autocorrelation, spectral analysis) and bivariate (cross-correlation, cross-spectral analysis) methods. Univariate methods characterise the structure of an individual time series (hydrograph), while bivariate methods describe the transformation of an input function into an output function (rainfall/discharge).

The Auto-Correlation Method is a tool for the identification of some overall characteristics of a discharge time series, particularly cyclic variations (Mangin 1982, Grasso and Jeannin, 1994, Eisenlohr *et al.*, 1997b). Spectral analysis offers considerable potential as a powerful tool for the analysis of periodicities within the time series (Box and Jenkins 1976, Mangin 1984).

Cross correlation methods permit the quantitative comparison of rainfall with spring discharge time series. The technique provides information about strength of the relationship between the two time series and the time lag between them (Jenkins and Watts 1968, Box and Jenkins 1976, Mangin 1981, 1982, 1984, Padilla and Pulido-Bosch 1995, Larocque *et al.,* 1998, Grasso 1998, Grasso and Jeannin 1998).

In the case of linear and steady state systems, a transfer function can be defined, which is a characteristic function of the system that reflects the active processes. The method of identifying the transfer function of an unknown system is known as deconvolution. Assuming the input function to be random, the cross-correlogram can be considered as the transfer function of the system (Neuman and De Marsily 1976, Dreiss 1989, Mangin 1984).

Discharge time series may exhibit non-stationarity in their statistics. While the series may contain dominant periodic signals, these signals can vary both in amplitude and frequency over long periods of time. One possibility for the analysis of non-stationarity of a time series would be to compute a Windowed Fourier Transform using a certain window size, and sliding it along the series. This would provide information on frequency localization, but would still be dependent on the window size used, resulting in an inconsistent treatment of different frequencies (Torrence and Compo 1998).

Wavelet analysis introduced by Grosmann and Morlet (1984) attempts to solve this problem by decomposing a time series into time/frequency space simultaneously. The continuous wavelet transform is defined as the convolution of the time series with a scaled and translated version of a wavelet function (Torrence and Compo 1998). Several types of wavelet functions are in use; each must have zero mean and be localised in both time and frequency space.

Wavelet analysis constitutes an alternative in karst hydrology to spectral and correlation analyses. By varying wavelet scale and sliding the wavelet along a time series, one can construct a picture showing both the amplitude of any periodic signals versus scale and the variation of this amplitude with time. A theoretical explanation of wavelet analysis is provided by Daubechies (1992). The application of wavelets in karst hydrogeology is discussed in Labat *et al.* (1999 a, b, 2000).

DISTRIBUTIVE METHODS

The spatial heterogeneity of karst aquifers may require the discretisation of a hydrogeological system into homogeneous subunits, each with its own characteristic hydraulic parameters. The discretisation of a rock volume involves the discretisation of the differential equations describing groundwater flow. The principal formula representing transient groundwater flow in saturated medium is the classical diffusivity equation derived from Darcy's flow law (momentum conservation) and the continuity equation (mass conservation):

$$S\frac{\partial H}{\partial t} = \nabla(K\nabla H) + i \qquad (4)$$

where S is the storage coefficient [L^{-1}], K is hydraulic conductivity [LT^{-1}], H is hydraulic head [L], t is time [T], and i is the source term [T^{-1}].

Two types of discretisation methods are used widely in hydrogeology. The Finite Difference Method (FDM) consists of subdividing the model domain into rectangular cells. Partial derivatives are then approximated by simple differences between a given number of adjacent nodes located at the corners or in the centre of each cell. According to the Finite Element Method (FEM), the model domain is subdivided into an irregular network of triangular and/or quadrangular finite elements. The approximation of differential operators is analytical, and it involves integral quantities for each element. The FEM allows the combination of one-, two- and three-dimensional elements of various shapes, thus facilitating proper discretisation and the implementation of discrete features into the model. A detailed explanation of the FDM and FEM discretisation methods can be found in Kinzelbach (1986), Wang and Anderson (1982), and Huyakorn and Pinder (1983).

The equation system obtained by spatial discretisation requires matrix inversion. This can be easily computerised, and thus spatial discretisation allows groundwater flow simulations in complex hydrogeological systems. The solution of an equation system requires the definition of boundary conditions and (for transient problems) initial conditions. Boundary conditions consist of the definition of either hydraulic potential (head boundary) or flux (flux boundary) values along the domain boundaries. Initial conditions consist of the spatial distribution of the unknown function, and are usually taken from an equipotential map or a previous steady state simulation.

Distributed parameter groundwater flow models include two principal concepts. The discrete concept considers the flow within individual fractures or conduits. In contrast, the continuum concept treats heterogeneities in terms of effective model parameters and their spatial distribution. These concepts can be combined into five alternative modelling approaches, according to the geometric nature of the conductive features represented in the model (Teutsch and Sauter 1991, 1998) (Figure 7):

- Discrete Fracture Network Approach (DFN)
- Discrete Channel Network Approach (DCN)
- Equivalent Porous Medium Approach (EPM)
- Double Continuum Approach (DC)
- Combined Discrete-Continuum (Hybrid) Approach (CDC)

The physical parameters of the flow medium can be directly or indirectly derived from real field observations (deterministic models) or can be determined as random variables (stochastic models). Each method has its respective advantages and limitations, and the selection of the appropriate modelling approach may be crucial with respect to the outcome of the simulation.

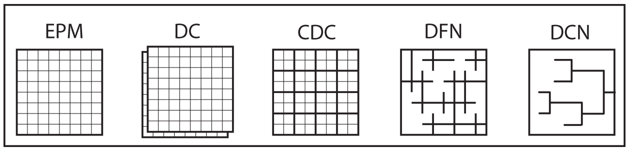

Figure 7: Classification of distributive karst modelling methods.

Discrete Fracture Network Approach (DFN)

According to the DFN approach, only certain sets of fractures are considered to be permeable. The matrix medium is assumed to have negligible permeability. This concept simplifies a fissured system into a network of two-dimensional fracture planes (Figure 8). It is mainly applicable to fractured aquifers. However, DFN methods facilitate the representation of karst channels by introducing one-dimensional elements or increased transmissivity zones along individual fractures representing dissolution voids (Dershowitz *et al.,* 2004).

The transmissivity T [L^2T^{-1}] of a single fracture can be expressed by the "cubic law" (Snow 1965) as follows:

$$T = \frac{a^3}{12}\frac{\rho g}{\mu} \tag{5}$$

where a is the fracture aperture [L], μ is the dynamic viscosity of water [$ML^{-1}T^{-1}$], ρ is fluid density [ML^{-3}], and g is acceleration due to gravity [LT^{-2}]. The "cubic law" is valid for laminar flow in open or closed fractures (Witherspoon *et al.,* 1980). The storativity of a single fracture assuming water compression only, can be expressed as follows:

$$S_f = \frac{\rho g}{E_w} a \tag{6}$$

The cubic law is based on a parabolic distribution of velocities between smooth parallel plates, which is a condition seldom met in natural fractures. Natural fractures are rough, tortuous and heterogeneous. The cubic law aperture can therefore significantly underestimates the storage and overestimates velocity. For this reason, it is preferable to derive fracture properties directly from field experiments. Packer tests provide information on transmissivity, interference tests estimate storage, and tracer tests determine transport aperture.

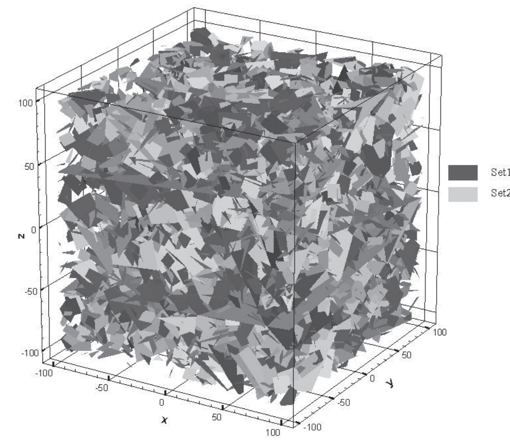

Figure 8: Example of a DFN network consisting of two fracture sets.

The first two-dimensional DFN modelling of synthetic fracture networks using a numerical solver was published by Long *et al.* (1982). Fracture networks were generated stochastically by applying a Monte Carlo process. Long *et al.* (1985) constructed a three-dimensional DFN model, and simulated steady-state flow for simple theoretical configurations of orthogonal fractures. Andersson and Dverstorp (1987) presented a modelling case study for conditioning the statistical generation of fracture sets with field data.

According to the Monte Carlo process, probability density functions are fitted to field data describing the spacing, length, direction and aperture of fracture sets identified on stereonets. Parameters are sampled from these statistical distributions, and assigned to individual fractures in the model. Fracture plane centres are generated first, assuming a negative exponential distribution of fracture spacing (Baecher *et al.,* 1977). Fracture sizes are then assigned and usually sampled from lognormal distribution functions. Fracture orientations usually show a Fisher distribution, while fracture transmissivities follow a lognormal distribution.

A major benefit of DFN models is their ability to reflect the compartmentalization phenomenon experienced at several study sites (Shapiro and Hsieh 1994, Sawada *et al.,* 2000). Compartments are adjacent rock volumes that manifest significant differences in hydraulic heads (up to 100 meters difference on a few meters of distance). Robinson (1984) demonstrated that there was a critical number of intersections required to produce percolating pathways. Compartments are the result of insufficient density of the fracture network. The porous medium concept assumes hydraulic continuity between all points in the simulation region, and cannot reflect compartmentalization phenomenon.

One of the most critical shortcomings of the DFN approach is its high data demand. While fracture spacing, orientation and transmissivity distributions can be estimated from downhole measurements, fracture size parameters usually remain uncertain. Computational constraints limit the number of simulated fractures to the order of 10^4 to 10^5. Fracture size distributions are usually truncated, and small size fractures are not represented in the model. This results in an erroneous estimation of the storage and diffusion behaviour, and necessitates the implementation of artificial matrix blocks in the model. As fracture parameters result from a stochastic generation method, model results involve significant spatial uncertainties.

Discrete Conduit Network Approach (DCN)

The DCN approach simulates flow in networks of one-dimensional pipes representing karst conduits or connections between fracture centres. A DCN conduit network can be established deterministically representing a local-scale field situation, or can be derived from stochastic DFN networks by geometric transformations.

The mathematical formulation of the average velocity of laminar flow in one-dimensional conduits may be expressed by the Hagen-Poiseuille law:

$$\vec{v} = -\frac{r^2}{8}\frac{\rho g}{\mu}\vec{I} \qquad (7)$$

where r is conduit radius [L]. Conduit conductance is a one-dimensional parameter derived from the Poiseuille law as follows:

$$K_c = \frac{\pi r^4}{8}\frac{\rho g}{\mu} \qquad (8)$$

The mathematical formulation of turbulent flow in one-dimensional conduits is given by the Darcy-Weissbach friction law:

$$Q = -K' A_c \sqrt{I} \qquad (9)$$

where K' is turbulent flow effective hydraulic conductivity [LT^{-1}], A is cross sectional area [L^2], and I is hydraulic gradient [-]. Louis (1968) expressed the effective hydraulic conductivity for fully constricted (phreatic) pipe flow as follows:

$$K' = 2\log\left(1.9\frac{D_h}{\varepsilon}\right)\sqrt{2gD_h} \qquad (10)$$

where $D_h=4R_h$ is the hydraulic diameter [L], and ε is the absolute size of irregularities along conduit walls [L]. Similarly, Strickler (1923) expressed the effective hydraulic conductivity for non-constricted (vadose) pipe flow as follows:

$$K' = K_s R_h^{2/3} \qquad (11)$$

where K_s is the Strickler coefficient [$L^{1/3}T^{-1}$] depending on roughness. R_h is the hydraulic radius (cross section divided by wet perimeter) [L]. The storage coefficient of a conduit assuming water compression only, can be formulated as follows (Cornaton and Perrochet, 2002):

$$S_c = \frac{\rho g}{E_w} r^2 \pi \qquad (12)$$

A summary and comparison of these formulae, along with case studies for their application is provided by Jeannin and Maréchal (1995), and Jeannin (2001). These authors constructed a two-dimensional fully constricted pipe-flow model of the Hölloch Cave, Muotatal, Switzerland, in order to simulate groundwater flow for various discharge conditions (Figure 9). The geometric properties of the conduits were derived from field observations. The model was calibrated on the basis of several head and discharge measurements in conduits. This study demonstrated how overflow conduits modify aquifer conductivity when they become active. This results in the observed large non-linearity of the system. The typical effective hydraulic conductivity of karst conduits ranges between 1 and 10 m/s and the Louis formula is adequate for calculating head-losses under such conditions.

Another application of the DCN approach is for the representation of fracture networks (Cacas *et al.*, 1990, Dverstorp *et al.*, 1992, Dershowitz *et al.*, 1998). Fractures are usually represent-

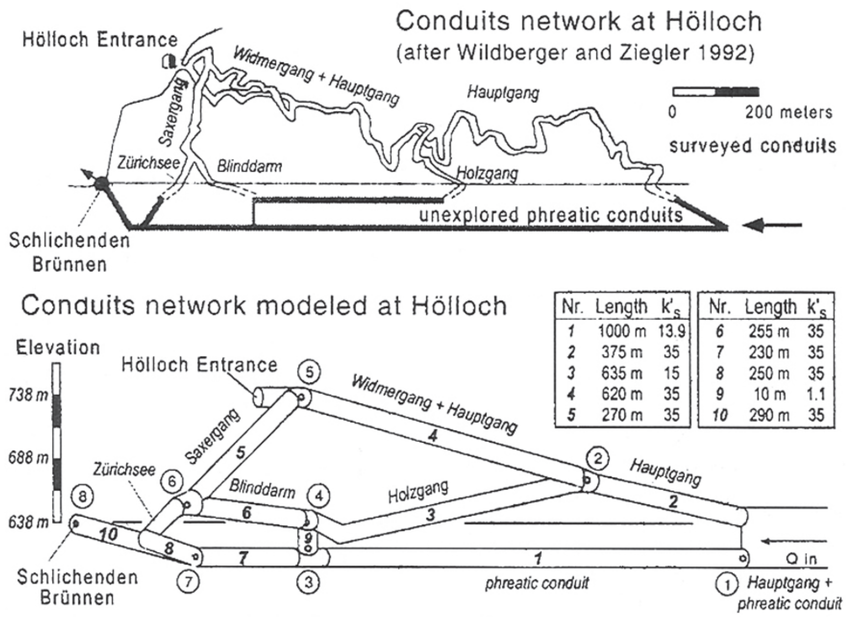

Figure 9: The DCN model of the downstream part of the Hölloch cave, Muotatal, Switzerland (Jeannin 2001). The parameter k's is equivalent to K'A.

ed by one-dimensional elements connecting fracture intersections. DCN representation of fracture networks may be useful when no spatial information on fracture flow is necessary. DCN networks derived from DFN models can correctly represent the overall transport behaviour of fissured systems, and can be used for simulating breakthrough curves. The transformation of fractures into one-dimensional pipes requires the estimation of effective pipe width, which remains a calibration parameter. The quality of DCN models in this case strongly depends on the quality of the DFN model from which they originate.

Equivalent Porous Medium Approach (EPM)

The EPM approach utilizes discretization units of similar size. This requires the representative elementary volume to be almost constant all over the model domain, and involves an insignificant change of aquifer hydraulic parameters between adjacent units of discretization. This is a condition seldom met in karst systems.

The substitution of strongly heterogeneous rocks with equivalent porous medium (this transformation referred to as upscaling) has long been an interest of hydrogeologists active in the field of fractured rock hydrology. Oda (1985) presented a method for calculating EPM properties from fracture networks. The Oda approach overlays an EPM grid across a fracture network, and derives EPM properties for each grid cell based on the fracture properties contained in that cell. The result is an equivalent permeability tensor, according to a specific grid. Fine discretisation reproduces the hydraulic and transport behaviour of the underlying fractured medium. However, for coarser practical discretisations of tens or hundreds of meters, the Oda approximation produces less accurate results.

The differences in transient hydraulic behaviour between fissured, karstic and equivalent porous systems were demonstrated by Kovács *et al.* (2005). These authors demonstrated that porous systems manifest fundamentally different temporal hydraulic behaviour from karstified medium. The global response of EPM models corresponds to the transition between matrix-restrained and conduit-influenced flow (Figure 10).

The application of EPM models for steady-state karst hydrogeological problems is mainly limited by the constraints of discretisation. Transient EPM models fail to reflect karstic hydraulic behaviour. As a consequence, the EPM approach is basically inadequate for modelling karst hydrogeological systems over a large spectrum of applications.

Double Continuum Approach (DC)

The difficulties of obtaining data for constructing DCN or CDC models, and the inability of the EPM approach to take account of the strong heterogeneity of karst aquifers, have motivated

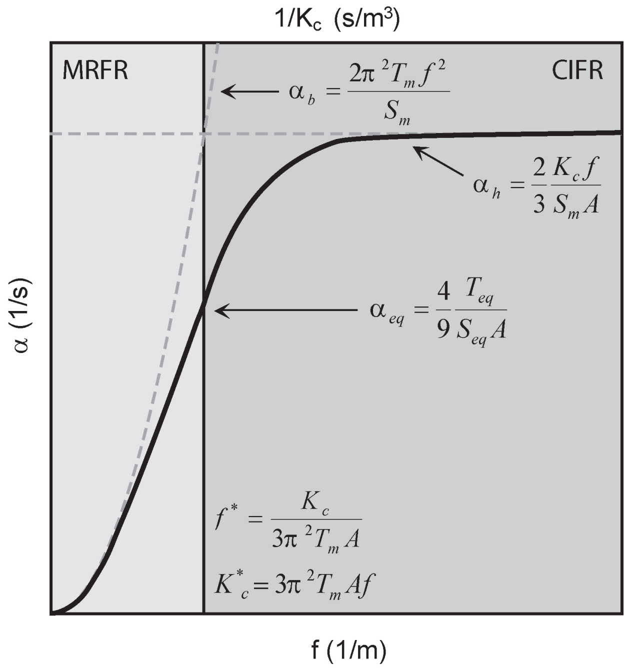

Figure 10: Variations of the recession coefficients for equivalent discrete-continuum models. EPM models correspond to the transition between matrix-restrained and conduit-influenced flow, and designates an inflection point of the recession coefficient curve. Modified after Kovács (2003) and Kovács *et al.* (2005).

the development of the DC method, which can simulate specific karst features without requiring detailed knowledge of the conduit network geometry. The first numerical solution using the DC approach was provided by Teutsch (1988), who used the original concept of Barenblatt *et al.* (1960) (Figure 11). In a double continuum model the conduit network and the fissured medium are both represented by continuum formulations. The exchange of water and solute between the two continua is calculated based on the hydraulic head difference between them, using a linear exchange term. Flow equations for the two separate media may be formulated as follows:

$$\nabla(K_i \nabla H_i) = S_s^i \frac{\partial H_i}{\partial t} + Q_i \pm \alpha_{ex}\left(H_1 - H_2\right) \tag{13}$$

Where i is the identifier of the medium considered, the last term is the exchange flux between the two continua, calculated from the hydraulic head difference, multiplied by an empirical steady-state exchange coefficient (α) as follows:

$$q_{ex} = \alpha_{ex}(H_1 - H_2) \tag{14}$$

The α parameter [$L^{-1}T^{-1}$] characterises the rate of the fluid transfer between the two media, which is composed of the hydraulic conductivity of the exchange zone and geometric factors. The flux value of a node (e.g. spring discharge) can then be evaluated as the sum of the fluxes from the two different media, while head values are observed separately in the two media.

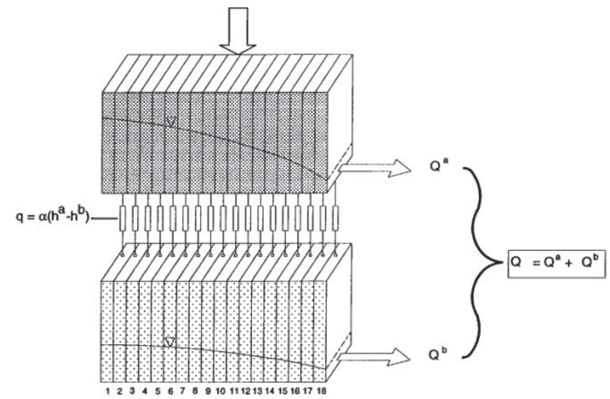

Figure 11. Schematic representation of a one-dimensional DC model (Teutsch 1988).

Although the DC concept can adequately describe the dual hydraulic behaviour of karst aquifers, applied hydraulic parameter distributions are volume averaged parameters of the real parameters. As the calibration of the parameters is basically a “trial- and-error” method based on available head data, the quality of the model results strongly depends on the density and location of the observation points. To some extent the parameters of the DC system can be estimated from pumping tests, especially for the matrix system. Sauter (1992) proposed parameter estimation methods for DC models.

The adequacy of DC model results and calibrated parameter fields has been tested by several authors. Mohrlok & Teutsch (1997) demonstrated that simulated spring discharges obtained from calibrated DC models show a good correspondence to field observations. Cornaton (1999) tested the physical meaning of the calibration parameter, and demonstrated a strong dependence of the exchange coefficient on matrix storage and conduit network density. The author also provided a one-dimensional analytical solution for the problem.

Another critical aspect of the DC concept is that it cannot handle the temporal delay of diffuse infiltration, since both subsystems are coupled directly at every node. In order to involve retarded diffuse infiltration in a DC model, a retention function is necessary (Sauter, 1992). Two-dimensional real-world applications of the DC concept were successfully performed by Teutsch (1988), Sauter (1992), and Lang (1995). Three-dimensional real-world applications of the DC method have not yet been performed because of the difficulties in model calibration and the necessity of requiring three-dimensional head data.

The DC approach is an effective tool for modelling karst systems. The spatial distribution of the calibration parameter values provides approximate information on real conduit system configuration. However, the interpretation of applied hydraulic parameters requires further attention.

Combined Discrete-Continuum (Hybrid) Approach (CDC)

The CDC approach is capable of handling the discontinuities that exist at all scales in a karst system (fractures, fault zones, karst channels, etc.), by representing them as embedded networks of different orders of magnitude. This nested model explains the duality of karst (duality of the infiltration process, the flow field and discharge conditions), and the scale effect on hydraulic conductivities (Király 1975).

Because the CDC approach uses the FEM discretisation method, it allows the combination of one-, two-, and three-dimensional elements (Király 1979, 1985, 1988, Király *et al.*, 1995, Király and Morel, 1976a). According to the CDC method, high conductivity karst channels can be simulated by one-dimensional finite elements, which are set in the low permeability matrix represented by three-dimensional elements. Similarly, the application of two-dimensional elements makes the simulation of fractures and fault zones possible.

The CDC method was developed and first applied by Király and Morel (1976a). This was the first time that the typical temporal behaviour of a karst spring was adequately simulated by a distributive model, using realistic hydraulic parameter distributions (Figure 12) Király (1998b) concluded that the application of EPM models requires artificially high hydraulic conductivities in order to simulate realistic hydraulic heads. However, these discrepancies disappear with the introduction of the high conductivity channel network into the FEM model. Typical spring hydrographs cannot be simulated without applying concentrated infiltration. The rate of this concentrated input should be more than 40 % of the total infiltration. The epikarst layer (Mangin 1975) may play an important role in draining and infiltrating water, and channelling it towards the karst network.

The CDC approach is the only distributive modelling concept that facilitates the direct application of observed aquifer geometry and measured hydraulic parameters. Consequently, the CDC approach facilitates the testing of conceptual models of karst systems. As demonstrated by Kovács (2003) and Kovács *et al.* (2005), the correct estimation of conduit network density and hydraulic parameters is crucial for modelling karst systems by the CDC approach. There is only one single parameter configuration that yields appropriate transient model results. These authors also demonstrated, that epikarstic storage can significantly influence the global reactions of a karst system, and thus such systems require the separate estimation of epikarst hydraulic and geometric parameters.

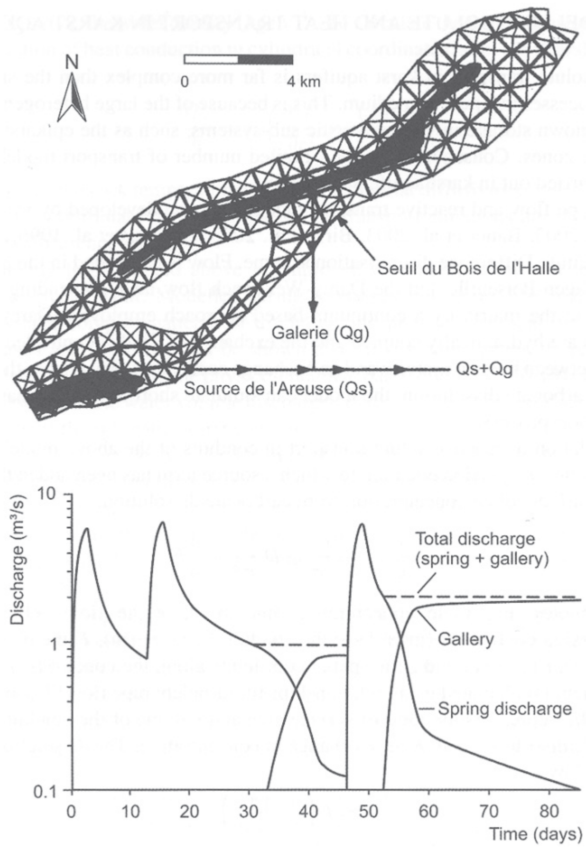

Figure 12. Finite element mesh and simulated spring hydrographs of the first CDC model (Areuse Basin, Switzerland), Király and Morel (1976a).

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Geochemistry and Climate Change

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INTRODUCTION

Karst terrains are developed on soluble rock and typically contain vital water resources. Over a range of time scales, chemical and clastic sedimentary deposits accumulate in karst terrains. These deposits can provide records of past environments, including climate and hydrologic change, which have been shown to be of particular importance to other sciences and to social policy and planning. This paper reviews the state-of-the-art and prospects for future research on three different but related facets of the geochemistry of karst terrains: (i) The application of geochemistry to understanding the hydrogeology of karst aquifers, and (ii) the geochemistry of speleothems and other karst sedimentary deposits as records of past environmental change over different temporal scales that are preserved in these deposits, and (iii) climate change questions that may be addressed by speleothem studies.

HYDROGEOCHEMISTRY

Naturally-occurring and introduced chemical tracers have been applied over the past several decades to advance our understanding of the hydrologic functioning of karst aquifers. In the future, karst water resources face increased impacts, both in terms of water supply demand and water quality, due to climate change and increases in population, urbanization, soil loss and other land use changes. It will therefore become increasingly important to supplement conventional tracing approaches that use dyes, temperature, specific conductivity, ion concentrations and stable isotopes of H, O and C, via innovative ways to apply these conventional geochemical tracers and by developing new tracers based on rare-earth elements, microbes and bacteriophages, stable isotopes of N S, and Fe, radiogenic isotopes of Sr, Nd, U, Th and Pb, naturally-occurring particulate materials, chlorinated and fluorinated compounds, and hormones, pharmaceutical and personal care products.

Challenges

Karst aquifers pose many challenges to understanding the complexities of their unique triple-porosity structure and tracing flow from recharge through infiltration to the different diffuse, fracture, and conduit flow paths within individual aquifers. In addition, the critical vulnerability of karst water resources to contamination due to their typically thin soil cover and discrete recharge features underscore the importance of addressing these challenges. Geochemical tracing of fluid flow paths in these systems is needed for testing and refining physical flow models. Among the major issues faced by karst researchers and water resource managers are (i) determining the sources of contaminants to groundwater and surface water; (ii) constraining the sites, timing, and amount of recharge to karst aquifers; (iii) defining and quantifying groundwater-surface water interactions, and (iv) understanding the timing and thresholds of urbanization impacts.

Prospects

Among the prospects for meeting the challenges outlined above are a number of naturally-occuring geochemical tracers, as discussed below.

Tracing flow paths: Identifying conduit flow paths in karst flow systems has been traditionally approached by different dye tracing approaches. Deconvoluting the interaction between the conduit and diffuse end-members in a karst flow system can be aided by geophysical (Vouillamoz *et al.*, 2003), biological (e.g., Rossi *et al.*, 1998; Mahler *et al.*, 2000), and geochemical tracing approaches. Geochemical approaches include the analysis of (i) isotopic and chemical tracers that have distinct compositions in surface runoff, soils and aquifer bedrock, as this provides a measure of the extent of water-rock interaction within different reservoirs, and therefore a measure of the extent of interaction with soils and rocks, and of residence times in the soil zone, in the conduit network, and in the matrix (Banner *et al.*, 1996; Uliana and Sharp 2001; Redwine and Howell, 2002; Celle-Jeanton *et al.*, 2003; Wilcox *et al.*, 2004); (ii) isotopic and chemical tracers with distinct compositions during periods of high vs. low recharge (Lakey and Krothe, 1996; Lee and Krothe, 2001; Jones and Banner, 2003), and (iii) the sediment dynamics in a karst system (Bottrell *et al.*, 1999). Time series of geochemical tracer data are useful in karst for examining signals and responses of hydrologic events using methods such as breakthrough curve decomposition, frequency distribution analysis, and lumped-parameter models. (e.g., Dreiss, 1983; Larocque *et al.*, 1999; Labat *et al.*, 1999; Bouchaou et al., 2002). Such approaches can be complemented by using geochemical tracer methods in conjunction with lumped-parameter, physics-based, and/or mathematical (e.g. stochastic-based) hydrological modeling (Dreiss, 1983; 1989; Bonacci 1993; Eisenlohr *et al.*, 1997; Halihan and Wicks, 1998; Jeannin, 2001; Long and Putnam, 2004). These methods are useful for understanding the complex dynamics of karst systems and quantifying the different flow components (Figs. 1, 2). There is significant potential for innovative approaches using these methods in karst.

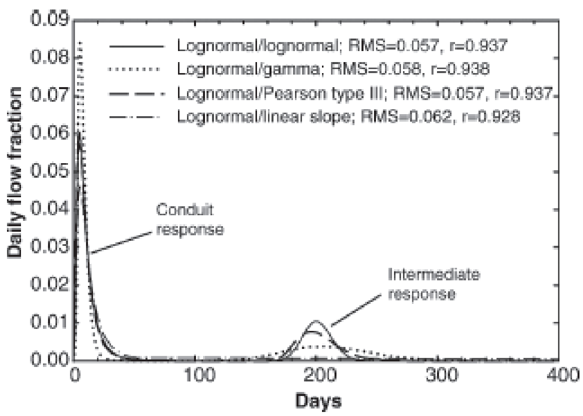

Figure 1. Transfer functions for well HH in the Madison Aquifer, South Dakota, representing combined conduit and intermediate components. All four transfer functions use a lognormal distribution for the conduit component and one of four distributions for the intermediate component, which includes lognormal, gamma, Pearson type III, or linear slope. The vertical axis represents the fraction of flow for each time step in days for the combined conduit and intermediate flow. From Long and Putnam (2004).

Groundwater age constraints: The distribution of groundwater age in a sample can be revealing regarding an aquifer's properties. The combined use of (i) multiple methods that constrain groundwater ages, such as ^{3}H, ^{14}C and CFC's, and (ii) new approaches to model the age information, has much potential (Long and Putnam, 2004; in prep.; Fig. 3). Such approaches permit examination of how changes in the age distribution of groundwater over time can reveal aquifer dynamics such as the interaction of conduit and diffuse flow components.

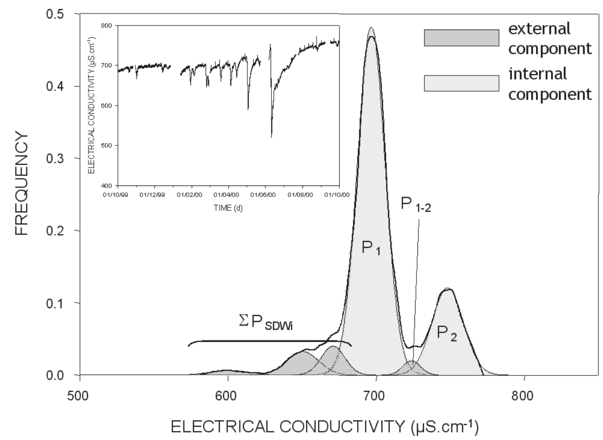

Figure 2. Conductance frequency distribution for Barton Springs for 2000 (bold line) and the underlying normally distributed populations (light lines). Time series of specific conductance (electrical conductivity) are shown as inset. From Massei *et al.* (2007).

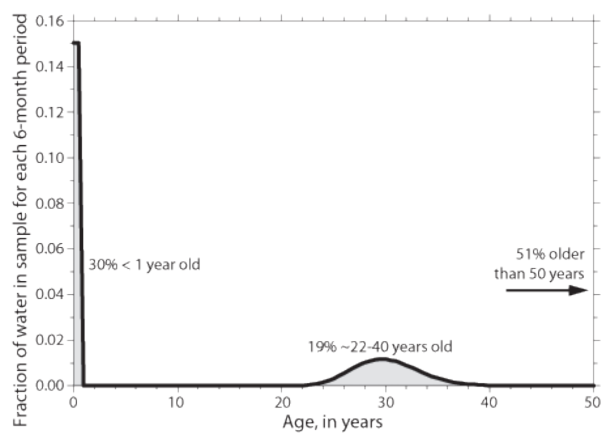

Figure 3. Probability density function of groundwater age from well RC-11 in the Madison Aquifer, South Dakota, using average parameter values calibrated for 1993-2005 (from A.J. Long and L.D. Putnam, work in progress).

Determining contaminant sources and using contaminants as tracers: Building on an improved knowledge from tracing groundwater flowpaths, a multi-parameter approach will become invaluable for determining the sources of contaminants to karst flow systems. This will involve the combined use of dye-tracing studies, natural- and labeled-sediment tracers (Mahler *et al.*, 1998), environmental tracers (e.g., nutrients, isotopes, CFC's, and bacteria; e.g., Mahler *et al.*, 2000), pharmaceutical compounds and personal care products, and geophysical data to identify contaminant sources and pathways of contaminant transport in ground water, spring water, and surface water. Adding to this arsenal, as analytical methods are advanced, will be microbiological indicators as new tracers to track contaminant sources and movement in ground water from waste disposal and water reuse.

In contrast to determining sources of particular contaminants, some contaminants already in the environment may be used effectively as tracers. Contaminants such as herbicides, insecticides and organic solvents offer significant advantages over introduced artificial tracers. They represent the actual contaminants of interest, typically are inadvertently introduced into the system at the same time at multiple points, and they may be introduced into the karst aquifer from a soil zone source during storms, when contaminant transport is of the most interest (Fig. 4).

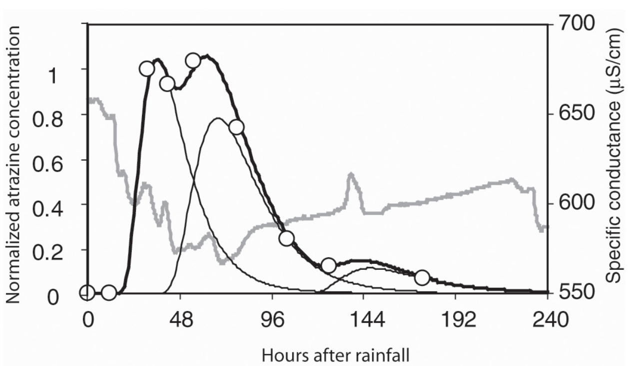

Figure 4. Decomposition of atrazine breakthrough from Barton springs, Austin, Texas, in response to a storm. Atrazine breakthrough (data shown as open circles) is modeled as the sum (bold line) of three log-normal distributions (fine lines) representing three different inputs of atrazine. Peaks in the individual breakthrough curves align well with troughs in specific conductance (grey line). From Mahler and Massei (2006).

Groundwater management: It would be of great benefit to groundwater managers and environmental consultants to have improved information about karst systems, such as (i) the timing, seasonality, and spatial distribution of recharge, (ii) the role of epikarst in storage and recharge to the karst aquifer (Bonacci, 2001), (iii) the impact of brush clearing and deforestation on recharge, (iv) estimating aquifer yield (Bacchus, *et al.*, 2003), (v) groundwater-surface water interactions, and (vi) aquifer response to anthropogenic impacts and climate change. Within the hydrologic community, advanced computer modeling and numerical simulation programs are available to efficiently and objectively estimate model parameters. These tools are difficult to apply, however, to karst systems due to the extreme heterogeneities in recharge and the subsurface, which make the estimation of the uncertainty in these parameter estimates difficult, if not impossible. If such models can be advanced to overcome these uncertainties, then application of these techniques may be useful for more efficient objective quantification of karst aquifer properties and data processing, as well as for advanced decision support systems in karst aquifers (Pierce *et al.*, 2005).

Using multiple geochemical indicators to estimate the timing and amount of mixing of water from the epikarst with ground water in shallow and deep parts of karst aquifers will be particularly important for public water supply wells. Tracers that vary in composition seasonally, such as H and O isotopes, can be used to determine the amount and extent of seasonality of recharge (Jones *et al.*, 2000; Andreo *et al.*, 2004). Development of more extensive sampling/monitoring networks that quantify surface water, recharge and subsurface flow will allow such methods to be applied on an aquifer- or watershed-specific basis. Innovative application and statistical analysis of tracers as conventional as conductivity have the potential to delineate storm-flow from pre-storm water and to separate water types into different modes of aquifer functioning and response to climatic variations (Fig. 1).

Impacts of urbanization: Geochemical tracers have great potential for quantifying the sources of urban water to karst and the impacts of urbanization on karst systems. Urbanization has been variably proposed to divert or concentrate recharge, via increased runoff, and, in contrast, to increase recharge, through leaking urban infrastructure (Krothe *et al.*, 2002; Pierce *et al.*, 2004). The impacts of land use change and urbanization on contaminant vulnerability (nutrients such as nitrate, pesticides, volatile organic compounds, pharmaceuticals and personal care products) and the non-linearity of processes within karst systems require that the thresholds of these impacts be understood. Geochemical time series that delineate the onset and extent of these impacts relative to the extent of urban development can be used to (i) identify impact thresholds and (ii) delineate between the contrasting effects on recharge. Such time-series data may come from recent karst spring deposits such as travertine (e.g., DeMott *et al.*, 2006).

Research Infrastructure Needs

Analytical advances in terms of isotopic analysis of specific organic compounds in water, portable chemical methods such as laser-induced breakdown spectroscopy (Harmon *et al.*, 2005), Raman spectroscopy and mini-differential optical absorption spectrometry can be applied to the *in-situ* monitoring of karst flow systems and the deposits that form therein. To the extent that the controls of environmental and anthropogenic factors on geochemical variations of modern karst systems can be understood, the more accurate will be interpretations of the geochemical records preserved in karst deposits (e.g., Tooth and Fairchild, 2003), as discussed in the next sections.

SPELEOTHEMS AS PALEOENVIRONMENTAL RECORDS

Caves are geographically widespread features within karst landscapes and provide unique preservation environments for deposits that contain proxy records of past environmental change. Speleothems – the chemical precipitates that form in caves – are especially important repositories for many different types of geochemical information. Research in the 1970s and 1980s demonstrated clearly that speleothems can be dated and contain information, which under certain circumstances, can be used to infer the conditions of their environment of deposition (Hendy, 1971; Thompson *et al.*, 1975; Schwarcz *et al.*, 1976; Harmon *et al.*, 1978; Gascoyne *et al.*, 1983; Yonge *et al.*, 1985). More recent research undertaken during the past two decades has illustrated that variations in the isotopic and chemical signal contained in speleothems can be used to reconstruct past changes in karst terrain hydrologic behavior as well as surface vegetation, weather, and climate change over different spatial and temporal scales.

The primary speleothem mineral is calcite, $CaCO_3$, but its metastable polymorph aragonite also occurs in the cave environment. In addition, other carbonate and sulfate minerals, such as gypsum, $CaSO_4{\cdot}2H_2O$, can be found in some cave environments. Less common minerals are found in special cave environments, usually in small amounts (Hill and Forti, 1997). Carbonate and sulfate minerals take on a variety of forms, but stalagmites, stalactites and flowstones are the most useful environmental archives.

Rainfall, with an H- and O-isotope signature determined by its origin and transport history in the atmosphere, falls on the karst land surface and infiltrates into the epikarst zone. With a variable time delay that is dependent on the complexity of the subsurface flow path, the water then percolates through the vadose zone until it intercepts an underlying cave. Chemical equilibration and ion exchange in the soil, chemical reactivity at the soil-bedrock contact, and the subsequent physical and chemical reactions occurring during infiltration through the limestone and during calcite precipitation once the seepage water enters the cave ultimately determines the trace chemical and isotopic composition of a speleothem. Of particular importance is the stable isotope exchange between cave atmosphere and drip water that occurs during calcite precipitation. Water dripping from the cave ceiling degasses CO_2 and deposits $CaCO_3$ as stalactites and then stalagmites and flowstone as the water subsequently reaches the cave walls and floor. Stalagmites grow at rates in the range of tens to hundreds of μm/year, so that a 1-m long stalagmite may represent a deposition time of 10^4-10^5 years. Age profiles of stalagmites can best be determined by U-series geochronologic techniques, although other dating techniques have been applied to speleothems. Axial profiles of stable isotope composition (e.g., calcite $^{18}O/^{16}O$ and $^{13}C/^{12}C$ and fluid inclusion D/H and $^{18}O/^{16}O$), trace element abundances, calcite color and luminescence banding, and organic acid content can provide robust information about the environment of present and past speleothem deposition as well as surface climate on local, regional, and global scales.

Challenges

There are several research needs and challenges related to understanding the formation of speleothems in caves and the use of the geochemical information contained in speleothems. The presence of humic, fulvic, and other organic acids has been documented in speleothems and are the main source of calcite color and luminescence. Depositional controls on organic compounds in speleothems are not well known and the paleoclimate significance of variations in organic compound abundance and composition in speleothems remains to be established. Variations in speleothems of trace elements such as Mg, Sr, Zn, Mn, Pb and U are well documented, but their potential as monitors of environmental change are yet to be fully determined. Another important need is an improved understanding that will lead to improving the models, or ‘transfer functions’, through which the parameters measured in speleothem calcite are translated into first-order climate information (e.g. variations in speleothem O-isotope ratios to surface temperature changes or C-isotope composition to surface vegetation type). In addition to speleothems, clastic sediment deposits also have potential to be dated (Schmidt, 1982; Granger and Muzikar, 2001; Granger *et al.*, 2001; Anthony and Granger, 2004) and provide useful paleoclimate information (Zhang, 1998).

Prospects

Caves are widespread features in karst terrains and speleothems are common cave features. Therefore, once fully understood, speleothem geochemical records have the potential to become the most important paleoenvironmental archive for carbonate islands and terrestrial domains, particularly for continental interiors.

Research Infrastructure Needs

There also are specific infrastructure needs that would facilitate the use of speleothems as paleoenvironmental records. Most critical is a formal system for access to state-of-the-art U-series geochronology because, at present, the number of age determinations needed by the research community is stressing the informal collaborative network that has been providing such support over the past decade. A central database of speleothem paleoenvironmental data would help to elucidate climate patterns at the regional or continental scale at different times. An archive for speleothems used for paleoenvironmental study would support the conservation of these rare materials, facilitate community access to studied samples for other types of analysis, and preserve these rare materials for future use as new analytical techniques emerge.

CLIMATE CHANGE

Speleothems can provide records of past cave depositional environment, karst aquifer hydrologic flow regime, and surface vegetation character, weather, and climate. Among the proxies in speleothems that are used in this regard are growth timing and rate, calcite stable isotopes (C and O) and radiogenic isotope (Sr and U) compositions, trace element variations, and fluid inclusion isotopic (H and O) composition.

Challenges

Understanding the temporal variability of water flow and storage in karst systems is needed over a range of time scales, including (i) seasonal to decadal scales, which are important for managing water resources, water quality and water availability, and (ii) century to millennial time scales, which are important for reconstructing hydrologic and climate change and their interactions. One challenge is constructing records for key time periods including the last glacial and interglacial maxima, intervals of abrupt change such as the Pleistocene-Holocene transition, the Younger Dryas, and the Little Ice Age. In addition, records are needed that cover the last century, to coincide with instrumented climate records and as a basis of predicting near-term future trends. An appropriate spatial coverage of climate records is needed in key geographic areas that drive and those that respond to important climate phenomena, such as the El Niño-Southern Oscillation and the seasonal monsoons. As with all proxy records of past change, valid interpretations of speleothem records are required. For speleothems, it is necessary to determine if equilibrium isotope and trace element precipitation occurs during the precipitation of individual calcite layers. Although recognized by early workers (e.g. Hendy, 1971), this has been a continuing and underappreciated challenge for speleothem studies (Mickler *et al.*, 2004, 2006). In short, the challenges described here may be summarized by the question: Can speleothems serve as equivalents of ice cores and deep-sea sediment cores as records of environmental change for tropical, temperate, and ice-free high-latitude terrestrial domains?

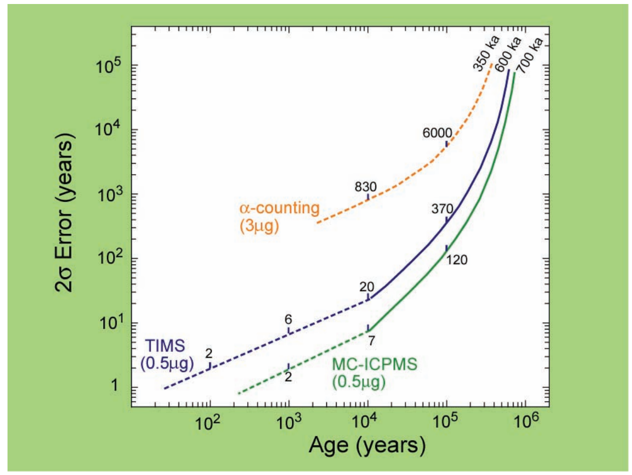

Figure 5. Comparison of analytical precision, ^{230}Th age, and sample size (in quantities of ^{238}U) for different U-series analytical techniques; α-spectrometry, thermal ionization mass spectrometry, and inductively-coupled mass spectrometry. The main reason for the improvement is the increase in ionization/transmission efficiency by about an order of magnitude (to more than 1%). The lines are calculated on the basis of counting statistics, but it has been demonstrated that the calculated precisions are possible on real samples. (From the work of R.L. Edwards, 2007).

Prospects

Geochronology: Any record of past environmental change, be it contained in a karst deposit, a marine sediment record or in an ice core, is only as good as its temporal resolution. It follows that accurate and precise geochronologic methods are the key to developing useful speleothem geochemical proxy records. In addition, non-geochemical variations such as the timing and growth rate of speleothem calcite can serve as an additional proxy if high-resolution chronologic data can be obtained. The advancing state-of-the-art is promising in this regard. Speleothem records can be continuous, spanning long periods of time, up to hundreds of thousands of years, and can be analyzed at high temporal resolution, often as high as decades and sometimes higher. Major improvements in analytical measurements of U-series nuclides took place two decades ago (Edwards *et al.*, 2003; Richards and Dorale, 2003), and additional improvements continue to occur (Musgrove *et al.*, 2001; Goldstein and Stirling, 2003; Edwards, 2007). Low porosity speleothem calcite that is free of detritus can be dated precisely and accurately by U/Th/Pa dating methods using modern mass spectrometry (Fig. 5). For less pure samples, geochronologic analysis of multiple speleothems from the same cave system can be used to develop convergent age models (Fig. 6). Some speleothems also exhibit repetitive banding, which can provide highly precise relative ages (Tan *et al.*, 2006).

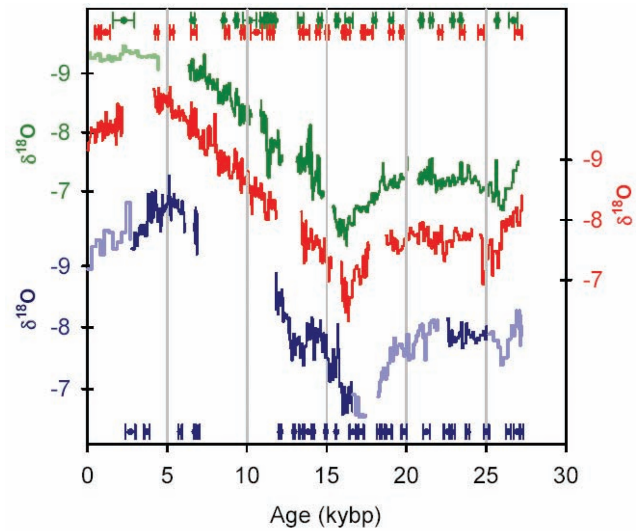

Figure 6. Three stalagmite records from Borneo. There were a total of five rounds of drilling samples and dating. After every round of dating, the stalagmite records continued to converge on a "master-record" which resembled the others. For this study, this required eliminating portions of records growing <10μm/yr (light portions on figure) (Partin *et al.*, 2007)

Key time-scales and regions: As noted above, paleo-records of seasonal-decadal and century-millennial resolution are needed. The research community should define several key geographic regions to be studied for a given climate mechanism and its impacts. For example, El Niño-Southern Oscillation phenomena are driven by sea surface temperatures in the Western Pacific Warm Pool, whereas its impacts occur in many regions around the globe, including the arid coast of Andean South America and Australia's drought-prone watersheds. Through the use of multiple proxies, such as speleothems, pollen, mollusks, corals, and tree rings) in a specific region and over a particular time interval, the different proxy records can be used to assess each other's efficacy and applicability.

Key time periods: In order to address some fundamental questions about climate change mechanisms, it is necessary to increase speleothem sampling resolution within and across the following climatic periods: glacial-interglacial transitions such as the Last Glacial Maximum and the Younger Dryas; inter-annual transitions and seasonality in the middle-late Holocene including modern and historical periods such as the Little Ice Age. The construction of records that cover the past century will increase the applicability of speleothem records to the current issue of global warming. Among the climate change mechanisms of interest are: long-term astronomical forcing and different recurrence intervals; variability in solar intensity; atmospheric-ocean oscillations (e.g., El Niño-Southern Oscillation, Pacific Decadal Oscillation, North Atlantic Oscillation); variability in temperature, precipitation, and monsoon intensity; and anthropogenic climate forcing.

Individual weather events: Among other measures of climate, the O-isotopic composition of cave calcite can provide a measure of fluctuations in the $^{18}O/^{16}O$ ratio of meteoric precipitation, an important variable that reflects the global climate state. For example, during tropical cyclone formation, intense rainfall will cause atmospheric water vapor masses to evolve to very low $\delta^{18}O$ values. An intriguing 20th century stalagmite record from Belize (Frappier *et al.*, 2007), sampled at very high spatial resolution (20 micron steps) reveals negative oxygen isotope excursions that coincide with the historical record of hurricanes in the Belize region (Fig. 7).

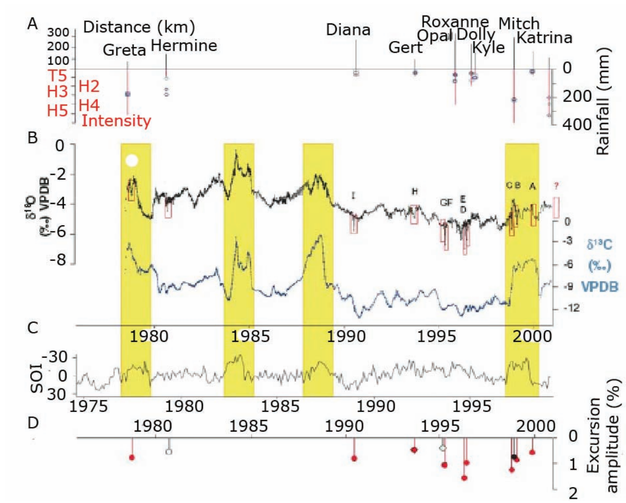

Figure 7. A Belizean stalagmite stable isotope record (B) and its correlation with recent tropical cyclone events in the region of Belize (A), and the Southern Oscillation Index (C). From Frappier *et al.*, (2007).

Abrupt climate change: Marine deep-sea sediment core and ice core paleoclimate studies have demonstrated that late Quaternary climate was dominated by abrupt climate change, geographically large and widespread shifts in climate taking place over just a decade (NRC, 2004). A significant societal concern stemming from this work is the possibility that greenhouse gas driven warming could trigger such an abrupt shift in the coming decades or centuries. Cave climate studies have the potential to provide valuable information on the spatial extent and causes of abrupt climate shifts in the past (Fig. 8).

Climate change summary: Several major contributions are expected from speleothem climate research. (i) A precise and accurate chronology for climate change for the last 600,000 years will be established. In the next several years, it is likely that climate records with chronologies precise to within a century will be obtained for events within the last 100,000 years. These chronologies will ultimately be based on concordant U-series dates for speleothems and correlations between speleothems and other types of proxy climate records. (ii) This line of research will yield a high-resolution picture of the temporal and spatial patterns of climate change and (in conjunction with ice core records) changes in atmospheric gas composition over the past several hundred thousand years. (iii) Based on these records, more comprehensive ideas will be formulated about the causes of climate change in the past, and by extension, into the future. The initial work in this field has demonstrated a strong connection between atmospheric composition and the glacial-interglacial cycles of the late Quaternary (Fig. 8).

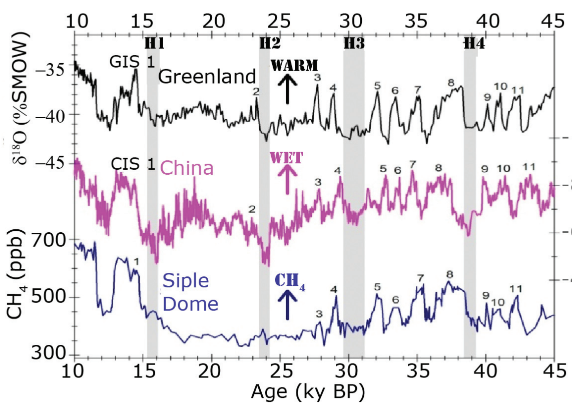

Figure 8. Correlation of Asian Monsoon, as recorded in a stalagmite from Hulu Cave in China, to ice core methane and oxygen isotopes, and to marine core Heinrich Events H1 through H4 (Wang *et al.*, 2001).

Research Infrastructure Needs

Evaluation and calibration of speleothem proxies, including the assessment of equilibrium precipitation of speleothem calcite, will be achieved through two main methods: (1) analysis of multiple proxies over a common time interval in the past; and (2) analysis of the modern system via monitoring networks in karst aquifers (Mickler *et al.*, 2004, Fig. 9). Understanding of temporal changes of the modern landscape above karst aquifers and the modern aquifer system are going to be essential for interpreting temporal records. This will require studies of changes 1) above caves (thickness, moisture, water chemistry, productivity of soils, vegetation, and weather, 2) at cave drip sites (cave-air meteorology, drip rate, drip composition, and 3) event sampling. Studies incorporating changes in land use driven by humans will make speleothem studies more relevant to human time scales, all independent of speleothem studies so that speleothem records may be better understood. This will require an even more extensive monitoring system and research infrastructure funding than that described above in the *Geochemistry* section.

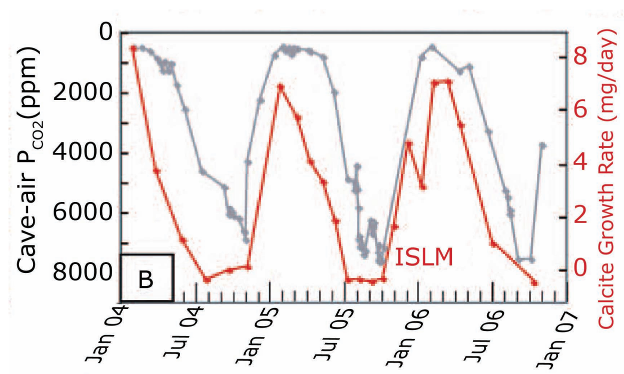

Figure 9. Seasonal variations in speleothem calcite growth on artificial substrates (red) and cave-air CO_2 (grey) in a central Texas cave. From Banner *et al.*, (2007).

The road to high-fidelity, well-dated speleothem paleoclimate records is paved with more dates than at first appearance are necessary (Fig. 6), and more reproducibility than is often funded/published. This will require laborious ultra-high-resolution reconstructions of 20th century climate and, absent visible banding, identifying chemical, isotopic, or petrographic proxies that occur in annual cycles, and counting back these annual cycles. The methodology for executing such records exists or can be developed, but it will require significant effort that can be driven by funding priorities.

To improve the accuracy of U-series age determinations, we will need an improved understanding of initial $^{230}Th/^{232}Th$ values incorporated into speleothems. Studies of modern system, zero-age speleothems, akin to studies of living corals to constrain marine initial $^{230}Th/^{232}Th$, will be valuable for addressing this issue.

In the next decade, this field of study will have a major impact in two areas of the earth sciences: (1) the understanding of the major factors that have caused the earth's climate to change in the past, and that likely will cause the earth's climate to change in the future, and (2) the understanding of those soil, vadose zone, cave, and climatic processes that affect the isotopic and chemical composition of karst waters and the cave deposits that precipitate from them. Important climate science goals will relate to the first point, whereas some significant understanding of the second set of processes will be necessary in order to fully realize the first set of goals. The improved understanding of the second set of processes will have direct application to important science questions regarding the operation of modern karst systems.

RELATION TO OTHER FIELDS OF STUDY

Other fields of study that karst research would have synergy with and bearing on are landscape ecology, microbial ecology; the global carbon cycle; biogeochemical cycles; water resource policy, contaminant flow and transport, and decision support systems.

IMPLICATIONS: CAVE CONSERVATION

The challenges are: Can we work in these environments without adversely affected them? Is it possible to enact an international research policy regarding cave research that scientists will follow? The varying factors of different local and federal restrictions on cave access and research permitting is an obstacle to be addressed. One aspect of such a policy might be the coring and reconnaissance analysis of speleothems vs. the multiple whole sample removal from caves. The karst community lacks an archival system for researchers such as that which exists for ice cores, yet speleothems are rarer than any individual ice core. There are competing pressures of multiple sampling trips needed if reconnaissance sampling is employed, vs. the time and funding required for the reconnaissance approach. One way to enact such a policy, if it could be agreed upon, would be to require a conservation plan as part of the "Broader Impacts" section of a research grant proposal.

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Caves and Karst as Model Systems for Advancing the Microbial Sciences

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INTRODUCTION

The subsurface is one of the Earth's major general habitats, defined as >8 m depth for terrestrial settings (e.g., Whitman, *et al.*, 1998). With millions of estimated microbial species (i.e. prokaryotic) occurring worldwide (e.g., Dykhuizen, 1998; Curtis *et al.*, 2002), the abundance of microbes in the subsurface could exceed numbers found elsewhere in the biosphere (Pace, 1997). However, our knowledge of the types and extent of subterranean life is poor, as is our understanding of the biogeochemical roles of microbes in the subsurface, due in part to limited accessibility and difficulty in obtaining uncontaminated material from the subsurface. Caves, representing discontinuous habitats that are characterized by complete darkness, nearly constant air and water temperatures, and relative humidity near saturation, allow for easy access to the subsurface from which samples can be taken and in situ experimentation is possible.

Caves can extend well below the 8 m depth, and link the surface to the subsurface. Early speleologists showed that microbes colonize caves, but their presence was predominately assumed to be as secondary degraders and food sources for higher organisms (e.g., Caumartin, 1963). Microorganisms in caves were considered to be merely a subset of surface, mostly soil, microbial communities, with just a few groups being potentially important for geological processes (e.g., Symk and Drzal, 1964; Hubbard *et al.*, 1986). Indeed, recent research has verified that cave and karst microbes are often translocated soil heterotrophs, chemoorganotrophs, or fecal coliforms that come into cave and karst systems from surface streams, on air currents, or carried into the cave by animal life, along with allochthonous organic matter and nutrient-rich sediments (e.g., Rusterholtz and Mallory, 1994; Mikell *et al.*, 1996; Grothand Saiz-Jimenez, 1999; Khizhnyak *et al.*, 2003; Simon *et al.*, 2003). Microbes can also be flushed into caves from meteoric drip waters (Laiz *et al.*, 1999), impacting the carbon load in nutrient depauperate ecosystems. However, because caves contain a range of physiochemical environments and habitats, and different physicochemical parameters dictate distinct metabolic strategies by microorganisms adapted to life in that particular set of parameters, there are also a wide variety of microbes that have been identified from caves and karst recently using culture- and molecular-based techniques.

One of the critical extreme conditions affecting most subsurface-adapted fauna, and the establishment and sustainability of subsurface, including cave, ecosystems is the lack of a continuous and rich nutrient supply, usually in the form of surface-derived (allochthonous) photosynthetically produced organic matter. In the absence of sunlight, however, redox-variable groundwater and reactive rock surfaces provide a wide assortment of potential energy sources that chemolithoautotrophic microbes can use to gain cellular energy. Some subsurface and cave ecosystems have been shown to rely exclusively on chemosynthesis (e.g., Stevens, 1997; Amend and Teske, 2005). The quality and quantity of chemosynthetic energy result in karst ecosystems teaming with life, from novel microbial groups (e.g., Engel *et al.*, 2003) to numerous endemic, cave-adapted metazoans (e.g., Sarbu e*t al.*, 1996; Culver and Sket, 2000; Hose e*t al.*, 2000; Sarbu *et al.*, 2000). The very recent discovery of the Ayalon Cave ecosystem in Israel, with eight invertebrates new to science and the possibility of a new chemosynthetically-based ecosystem, is testimony that substantial and unknown subterranean diversity still exists (Por, 2007)

From a deep time perspective, the impact of microbes on cave and karst habitats can be profound. Karst is ubiquitous worldwide (12.5% of the ice-free land surface), and carbonate rocks, accounting for ~20% of all Phanerozoic sedimentary rocks, form today and exist in 3.5 Ga deposits (e.g., White, 1988; Ford and Williams, 2007). Therefore, karst surface and subsurface microbial habitats have presumably existed throughout much of Earth's history. Moreover, because of physical constraints, caves and karst can be long-term reservoirs for microbial communities, as some systems remain relatively unchanged for thousands, if not millions, of years (e.g., Gale, 1992).

Considering the worldwide distribution of karst, and regardless of the public's fascination with "dark life" and unseen microbial worlds (e.g., Taylor, 1999), or of appreciating the sheer biodiversity of life on earth, it is imperative that we obtain a better understanding of the types of microbial communities in these systems, as well as what physicochemical and ecological conditions may be controlling diversity. These basic geobiological issues are critical to identifying how microbes alter karst and affect the productivity of karst aquifers and petroleum reservoirs (e.g., Hill, 1990; Palmer, 1995; Mahler *et al.*, 2000), and to preserving the integrity of karst through predicting changes that may occur following disturbance (van Beynen and Townsend, 2005).

In general, new molecular techniques developed in the 1980s and '90s increased the number of environments that could be successfully studied by microbiologists (Pace, 1997). These techniques revealed many microbiological processes and opened the door to examining the complex chemical interactions of microbial physiology with redox-active minerals (e.g., Newman and Banfield, 2002). In 1994, the Karst Waters Institute sponsored the "Breakthroughs in Redox Geochemistry and Geomicrobiology" (referred to as "Breakthroughs" herein) conference that brought together karst and non-karst microbial scientists from different biological and geological subdisciplines. The cross-fertilization had a profound effect and strong growth in the study of cave and karst geomicrobiology followed.

Characterizing the diversity of microbes in a habitat is important, but now, geomicrobiologists, microbial ecologists, and those interested in the microbial sciences, would like to accomplish at least one of two major tasks to understand the functional roles and distribution of microbial communities in that habitat: (1) culture as many microbes as possible, especially those with key geoecological functions, even though it is possible that a microbe may perform differently in the lab than in its natural habitat; or (2) characterize single cells using non-invasive, in situ methods, such as microscopy or other genomics and proteomics approaches (e.g., Cohen, 2001; Fernandez, 2005; Hong *et al.*, 2006; Wagner *et al.*, 2006). Broadly speaking, the field is much closer to attaining the second task than the first. Based on the previous and ongoing cave and karst microbiological investigations, most research is at the descriptive stage. Only a few species have been described from cultures, and only recently have non-invasive genomics methods been employed. By connecting phylogeny to function utilizing cultivation, genomics, and proteomics in the future, we will have an improved view of the ways that microbes shape their habitat and of the role that the cave and karst habitat has in selecting the metabolic and functional types of microbes within that habitat.

In this overview, we summarize the current knowledge base regarding microbial sciences of cave and karst settings, present basic research questions surrounding the study of microbes in caves and karst, and explore the relevance of these investigations to advancing disciplinary fields within the microbial sciences. We attempt here to recognize international efforts, but acknowledge that most of the review emphasizes North American and European work published in English. The foci of the review, in the context of caves and karst, are:

- Microbial biodiversity
- Microbes and ecosystem function
- Microbial roles in geochemical and geological processes

We conclude with possible applied aspects of cave and karst geomicrobiology research.

MICROBIAL BIODIVERSITY

All three domains of life (Eukarya, Bacteria, and Archaea), as well as viruses, occur as microscopic life in caves. From a purely diversity-centric standpoint, understanding who the microbes are, and how microbial communities relate to each other from one system to another, are important avenues for future ecological and geobiological research.

Culture-based methods are perhaps the most desirable, but genomic approaches have tremendous potential to explore the extent of biodiversity and to uncover novel diversity. Culturing is the biggest stumbling point for species descriptions because ~90-99% of microbes from any given habitat are unculturable. Without significant adaptation of culturing techniques and media, regardless of the potentially high numbers of species in that habitat, culturing diverse metabolic groups is difficult (e.g., Amann *et al.*, 1990; Hugenholtz *et al.*, 1998; Zengler *et al.*, 2002; Radajewski *et al.*, 2003; Schleifer, 2004; Stevenson *et al.*, 2004; Wagner *et al.*, 2006). In particular, culturing practices for cave and karst microbes require attention, as many of the habitats are oligotrophic (e.g., Cunningham *et al.*, 1995; Barton *et al.*, 2004). Culturing often introduces selective bias toward microbes able to grow quickly and which out-compete slow-growing organisms. But, culturing allows for quantification of metabolically active microbes and can lead to formal descriptions of species (e.g., Margesin *et al.*, 2004), as well as enhanced understanding of microbial byproducts that may be useful in applied research (e.g., pharmaceutical industry, bioengineered systems) (e.g., Herold *et al.*, 2005).

There have been numerous culture-based studies from cave and karst settings that resulted in the characterization of novel species and elucidation of the roles that the microbes may play in geochemical and geological processes (e.g., Brigmon *et al.*, 1994; Mikell *et al.*, 1996; Hose *et al.*, 2000; Vlasceanu *et al.*, 2000; Engel *et al.*, 2001; Lee *et al.*, 2001 ; Canganella *et al.*, 2002; Mulec *et al.*, 2002; Khizhnyak *et al.*, 2003; Laiz *et al.*, 2003 ; Gerič *et al.*, 2004; Margesin *et al.*, 2004; Bates *et al.*, 2006; Ikner e*t al.*, 2007). The rate of these studies compared to investigations using molecular techniques has slowed and is not comparable to the recent expansion of culture-based studies and descriptions of new microbial species from diverse habitats, especially from the marine realm.

We have been able to identify microbes that may be difficult, if not impossible, to cultivate using advanced genetic techniques. 16S rRNA gene surveys are one of the most common strategies used to characterize microbial communities, including from various caves and karst (Table 1). With the genetic data, microbial species diversity is evaluated, and dataset completeness and biodiversity indicators have also been determined. Many

Table 1: Summary of molecular investigations from caves and karst, differentiated by general habitat type. Many of these studies document the presence of novel species whose similarity to other known species or environmental isolate genetic sequences is relatively low (80-92% sequence identity).

Sulfidic Systems (e.g., microbial mats, cave-wall biofilms, cenotes)	Iron/Manganese Deposits	Oliographic/ Carbonate Systems	Other/General, including cave paintings
Vlasceanu *et al*. (1997)	Northup *et al*. (2000)	Cañaveras *et al*. (1999)	Lee *et al*. (2000)
Angert *et al*. (1998)	Boston *et al*. (2001)	Northup *et al*. (2000)	Lee *et al*. (2001)
Humphreys (1999)	Northup *et al*. (2003)	Holmes *et al*. (2001)	Schabereiter-Gurtner *et al*. (2002a)
Vlasceanu *et al*. (2000)	Chelius and Moore (2004)	Sanchez-Moral *et al*. (2003)	Schabereiter-Gurtner *et al*. (2002b)
Hose *et al*. (2000)	Spilde *et al*. (2005)	Barton *et al*. (2006)	Schabereiter-Gurtner *et al*. (2004)
Engel *et al*. (2001)		Cañaveras *et al*. (2006)	Laiz *et al*. (2003)
Canganella *et al*. (2002)		Galan (2006)	Zimmermann *et al*. (2005)
Engel *et al*. (2003)		Barton *et al*. (2007)	Barton *et al*. (2006)
Engel *et al*. (2004a)			Gonzalez *et al*. (2006)
Hutchens *et al*. (2004)			Weidler *et al*. (2007)
Barton and Luiszer (2005)			Northup *et al*. (2008)
Herbert *et al*. (2005)			
Macalady *et al*. (2006)			
Macalady *et al*. (2007)			
Meisinger *et al*. (2007)			

of the molecular studies listed in Table 1 resulted in the examination of bacterial and archaeal diversity in sulfidic and iron/manganese cave or karst habitats, with only a few studies probing oligotrophic and/or heterotroph-dominated habitats. Most of the studies were also conducted with the primary purpose of understanding geomicrobiological or geochemical processes, with limited attention to the potential source of microbes to the caves, or to biogeography and evolution issues.

In all practicality, microbes can not be characterized like metazoans, and classification is complicated by different levels of genetic relatedness and the extent of gene flow within and between taxa (i.e. horizontal gene transfer). This has resulted in a limited assessment of microbial biodiversity from caves, which impacts whether or not the biodiversity of karst should consider microbial "species". Although difficult to consider (at some level) how microbial species could be defined, it is generally agreed that a microbial species is a monophyletic and genomically coherent cluster of individuals with a high degree of similarity in independent traits (e.g., physiological, chemotaxonomical, and morphological) (e.g., Amann *et al.*, 1990; Hugenholtz *et al.*, 1998; Schleifer, 2004). Moreover, according to Cohen (2001), the traditional approach of species definition may not be particularly applicable to bacteria, and instead microbes within a species are better defined by ecotype, as a "set of strains using the same or similar ecological resources" (Cohen 2002). Clearly, 16S rRNA-based surveys alone are not adequate to assess the diversity of microbial communities due to the highly conservative nature of the 16S rRNA gene and because microbes with genetically identical 16S rRNA gene sequences can have quite distinct ecological function (e.g., Cohen 2002; Acinas *et al.*, 2004). Therefore, future work describing microbes from caves and karst should explore and emphasize non-rRNA (i.e. functional) gene diversity to answer fundamental questions relating to biodiversity. Cave and karst microbial communities may be particularly important in evaluating the microbial species and ecotype concepts because caves are globally distributed and have remarkably similar physicochemical constraints from cave to cave.

Even though these culture-based and molecular studies have gotten us closer to understanding the full diversity of caves, we still do not understand fully microbial metabolism in natural settings. Metabolic activity can only tenuously be implied from phylogeny, especially for novel or uncharacterized groups. Therefore, additional molecular techniques can identify metabolic activities much better (e.g., Radajewski *et al.*, 2003; Wagner *et al.*, 2006). An iterative process of gleaning information from phylogenetics studies to guide culturing efforts, followed by phylogenetic analysis of cultures that are displaying the physiological traits of the organisms, has been employed by some researchers (Spilde *et al.*, 2005). Molecular techniques paired with stable isotope systematics and isotope probing investigations are also useful. To date, there have been only a few isotopic studies (e.g., Pohlman *et al.*, 1997; Vlasceanu *et al.*, 1997; Humphreys, 1999; Vlasceanu *et al.*, 2000; Engel *et al.*, 2004a; Hutchens *et al.*, 2004), but future studies should focus on these types of complementary investigations. Moreover, whole gene amplification and metagenomics approaches are critical to characterizing the metabolism of cave and karst microbes, especially when culturing is not possible or successful.

MICROBES AND ECOSYSTEM FUNCTION

Most cave ecosystems generally reflect being energy- and nutrient-limited because of their dependence on allochthonous organic material. As such, the flux of microbes in cave streams or through epikarstic dripwaters can have a significant impact on the structure and dynamics of cave food webs (e.g., Laiz *et al.*, 1999; Gerič *et al.*, 2004). However, the discovery of chemolithoautotrophically-based ecosystems at the deep-sea hydrothermal vents toppled the dogma that all life on earth was dependent on sunlight. In 1986, Cristian Lascu, Serban Sarbu, and their colleagues found the uniquely diverse chemolithoautotrophically-based ecosystem from the hydrogen sulfide-rich (*sulfidic*) groundwater associated with the Movile Cave, Romania (e.g., Sarbu *et al.*, 1996). This discovery was extremely important to advancing cave and karst ecological and geomicrobiological research. Despite understanding the importance of chemolithoautotrophy, and knowing that microbes can contribute to ecosystem carbon and nutrient loading just by their presence (e.g. Pronk *et al.*, 2006), there have been relatively few studies that document the occurrence, distribution, diversity, and metabolic potential of microbial communities as applied to karst ecosystem function, such as for carbon cycling (e.g., Airoldi *et al.*, 1997; Simon *et al.*, 2003; Engel *et al.*, 2004a; Gerič *et al.*, 2004). For instance, a rich and abundant food source provided by chemolithoautotrophy may reduce nutritional stress to subsurface fauna because members of the ecosystem would not have to rely on outside food or energy (e.g., Howarth, 1993).

MICROBIAL ROLES IN GEOCHEMICAL AND GEOLOGICAL PROCESSES

The joint research efforts of geologists and microbiologists, and the growing number of geomicrobiologists trained in interdisciplinary fields, have promoted new scientific avenues, including the examination of what minerals or geological/geochemical processes could be considered biotic and what was abiotic. For instance, biologists have discovered that filamentous morphologies can be minerals, and geologists are discovering cellular morphologies in their microscopy investigations (e.g., Barton *et al.*, 2001). Important advances have been made in cave and karst geomicrobiology, but many challenges lie before us as we study the role of microorganisms in transforming and cycling nutrients and various compounds. Microbes, and specifically chemolithoautotrophs, can have a profound impact on geochemical and geological processes in karst, although the extent to which we currently can recognize and understand how microbes are a geochemical and geological force in subsurface settings is likely underappreciated. It is also not clear the extent to which microbes have impacted geologic processes through time, but caves and karst provide unique and accessible sites to explore these interactions.

Detailed accounts of previous creation of the cave and ongoing cave and karst geomicrobiological studies are included in Jones (2001), Northup and Lavoie (2001), Barton (2006), Barton and Northup (2007) and Engel (2007), so here we will emphasize only major accomplishments. Specifically, the highlighted studies demonstrate the progress that is being made in understanding the geomicrobiological and biogeochemical roles of microorganisms in metal and nutrient cycles, including iron and manganese, nitrogen, sulfur and carbon. As such, advances pertaining to carbonate precipitation and dissolution are also being accomplished.

Iron and Manganese Mineral Formation and Transformations

There has been considerable progress made in understanding the role of microorganisms in iron and manganese cycling in caves. In 1986, Peck (1986) suggested that chemolithoautotrophic microbes were primary producers in the iron and manganese deposits in which they were found. Cunningham and his collaborators were the first to recognize an association between microbial species and ferromanganese deposits within Lechuguilla Cave in New Mexico (Cunningham *et al.*, 1995). A variety of microbial groups were associated with the ferrihydrite stalactites, and microbes enhanced precipitation rates up to four orders of magnitude compared to inorganic processes (Kasama and Murakami, 2001). Northup *et al.* (2003) established the presence of a diverse community of microorganisms, some of whom were related to known manganese and iron-oxidizing bacteria and others who appear to be previously unknown. They also documented the presence of metabolically active bacteria in the punk rock underlying the ferromanganese deposits from Lechguilla and Spider Caves, New Mexico. Spilde *et al.* (2005) also demonstrated that some of the mineral species identified in those deposits can be reproduced in vitro by microbial species inoculated from these environments. There are archaeal sequences retrieved from this system and from the paleofill in Wind Cave, South Dakota (Chelius and Moore, 2004).

Additional forms of poorly crystalline manganese oxides, hydroxides, and carbonates (e.g. pyrolusite, romanechite, todorokite, and rhodochrosite) have been described from caves (Gradzinski *et al.*, 1995; Onac *et al.*, 1997; Northup *et al.*, 2000). In particular, irregularly shaped crusts of manganese flowstone (2 – 20 mm thick) are found in Jaskinia Czarna Cave (Tatra Mountains, Poland). Filaments and globular bodies are interpreted as bacterial or fungal cells that participated in the formation of the flowstone.

Nitrate Minerals and Nitrogen Cycling

Nitrogen is a limiting nutrient in most environments and a greater understanding of how microbes influence nitrogen cycling is critical to appreciating not only geochemical and geobiological roles, but also ecosystem functioning in karst (Stern *et al.*, 2003). At the 1994 "Breakthroughs" conference, George Moore wrote "When will we have an accepted explanation for cave nitrate deposits?" This statement captures the debate that has spanned more than a century. Despite the significance of cave nitrates in the early history of caves (i.e. for saltpeter deposits) there has been little work that has advanced our understanding and no consenss has been reached concerning the role of microbes during nitrate formation in caves. Studies evaluating microbial transformations of nitrogen compounds and directly testing mineral precipitation reactions and processes from cave and karst habitats, have not been done.

Specifically, researches still deliberate over the degree to which bacteria, such as ***Nitrosomonas*** spp. and ***Nitrobacter*** spp., participate in the creation of the cave saltpeter deposits, although ***Nitrobacter*** spp. had been cultured from nitrogen-rich cave sediments in Mammoth Cave (Fliermans *et al.*, 1974).

Sulfur Cycling and Transformations

There is a class of caves and karst that developed from waters rich in reduced sulfur compounds (mostly hydrogen sulfide), although sulfidic caves are generally rare (e.g., Palmer, 1991). Recently Engel (2007) and Por (2007) reviewed the hydrogeologic origin and ecology of these types of karst systems. Because many sulfur cycle transformations are catalyzed almost exclusively by microbes, sulfidic caves are important to study because of their rich biodiversity, variety of ecosystem function pathways, and unique hydrogeological processes that provide insight into groundwater and hydrocarbon reservoir development and evolution. These systems can also be used to proxy biogeochemical processes in Earth's history, as the speciation of sulfur compounds in microbial mats from active sulfidic caves was examined to understand sulfur isotope ratio systematics of ancient geologic materials (Engel *et al.*, 2007).

Previous research describing the microbiology of sulfidic caves and stratified systems, like cenotes, sinkholes, and anchialine (or anchihaline) caves, was based on microscopy or culturing (e.g., Hubbard *et al.*, 1986; Stoessell *et al.*, 1993; Brigmon *et al.*, 1994). But, genetic methods have significantly expanded the known microbial diversity from these systems (Table 1), and isotope systematics studies also unveiled possible microbial metabolic pathways and ecosystem function (e.g., Sarbu *et al.*, 1996; Pohlman *et al.*, 1997; Humphreys, 1999; Vlasceanu *et al.*, 2000; Engel *et al.*, 2004a; Hutchens *et al.*, 2004; Herbert *et al.*, 2005). Recent work in the Movile Cave, the Frasassi caves in Italy, and Lower Kane Cave in Wyoming suggests that chemolithoautotrophic sulfur-oxidizing bacteria generate energy that can sustain complex cave ecosystems (e.g., Sarbu *et al.*, 1996; Sarbu *et al.*, 2000; Vlasceanu *et al.*, 2000; Engel *et al.*, 2004a). Observational studies and phylogenetic analyses of 16S rRNA genes from microbial mats in these and other sulfidic caves indicate that similar microbial lineages are prevalent, including *Thiothrix* spp. and novel, presently unculturable *Epsilonproteobacteria* (e.g., Angert *et al.*, 1998; Engel *et al.*, 2001; Engel *et al.*, 2003; Engel *et al.*, 2004a; Barton and Luiszer, 2005; Engel, 2007; Macalady *et al.*, 2006). Future work should address the potential biogeographic significance of these microbes in caves and the subsurface as a whole.

Compared to neutral pH, carbonate-dominated, subaerial surfaces in caves without sulfidic waters, the walls and subaerial surfaces of sulfidic caves have been the foci of research because these surfaces often have cave-wall biofilms (snottites) with extremely acidic pH (0-4) (e.g., Hose *et al.*, 2000; Vlasceanu *et al.*, 2000; Macalady *et al.*, 2007). Interestingly, the microbial diversity of these biofilms, regardless of the cave, is similar phylogenetically and these extremophile microbial groups are exceedingly important to speleogenesis (Macalady *et al.*, 2007).

Carbonate Mineral Formation

Understanding biological induction and control on the formation of travertine and carbonate deposits has expanded our knowledge of the structure and formation of secondary carbonate deposits in caves (e.g., Léveillé *et al*,. 2000; Sanchez-Moral *et al*., 2003; Barton and Northup, 2007). Numerous studies of the petrographic fabrics of carbonate deposits coupled to stable isotope ratio analyses have been done to identify microfossils and microbially mediated minerals (e.g., Boston *et al.*, 2001; Melim *et al.*, 2001). Castanier and colleagues (1999) suggested that bacterial autotrophic processes cause depletion of carbon dioxide around cells, favoring carbonate precipitation. From enrichment culturing of microbes from speleothems in Cervo Cave in Italy, Cacchio *et al.* (2004) demonstrated using oxygen and carbon isotope ratio systematics that microbes were precipitating carbonate at unusually high rates compared to microbes from non-karst environments. By using a combination of petrographic analyses, cultivation, and molecular phylogenetics, Cañaveras and collaborators (1999; 2006) showed that moonmilk contained numerous filamentous species of *Proteobacteria* that caused calcite to precipitate after colonizing rock surfaces. Several studies have tried to link moonmilk formation to microbial processes (e.g., Gradzinski *et al.*, 1997; Mulec *et al.*, 2002; Galan, 2006), although some researchers found that microbes apparently did not play a role during formation in some caves (Borsato *et al.*, 2000). Recently, our knowledge of carbonate precipitation by microbes and our experimental sophistication is deepening due to experiments involving cultured microorganisms, including *Bacillus* spp. that mediated calcite precipitation under well-constrained laboratory conditions (Baskar *et al.*, 2006) and the characterization of genes in *Bacillus subtilis* that are involved in calcite biomineralization (Barabesi *et al.*, 2007).

Carbonate Dissolution

The classic, textbook, model for karst development involves carbonic acid dissolution, usually at shallow depths near the water table. However, carbonate rocks can also dissolve from reactions with other acids, including microbially produced organic acids and sulfuric acid (e.g., Adkins *et al.*, 1992; White, 1997; Perry *et al.*, 2004). The sulfuric acid speleogenesis model was proposed by Stephen Egemeier from his observations of Lower Kane Cave (1981). Nearly all of the subsequent investigations of sulfuric acid speleogenesis assumed that H_2S was oxidized to sulfuric acid abiotically, although the presence of microbes in these systems was alluded to have an influence (e.g., Ball and Jones, 1990; Hill, 1990; Hill, 1995; Sarbu *et al.*, 1996; Angert *et al.*, 1998; Hose *et al.*, 2000; Northup *et al.*, 2000; Sarbu *et al.*, 2000; Vlasceanu *et al.*, 2000; Engel *et al.*, 2001). The role of microbes in speleogenesis was reexamined in Lower Kane Cave, and the sulfur-oxidizing bacteria consumed nearly all of the H_2S in the cave waters, focused acidity on carbonate surfaces, and caused colonization-promoted dissolution (Engel *et al.*, 2004b). Research in other caves and sulfidic aquifers, including the Edwards Aquifer in Central Texas, has lead to a better understanding of the potential role of microorganisms during karstification and hydrocarbon reservoir modification (Barton and Luiszer, 2005; Macalady *et al.*, 2006; Randall, 2006; Macalady *et al.*, 2007).

APPLIED ISSUES SURROUNDING CAVE- AND KARST-RELATED GEOMICROBIOLOGICAL STUDIES

The presence and activity of microbes in caves and karst may yield beneficial byproducts, such as antimicrobial agents, surfactants, and enzymes, that can be used in applied sciences. Because limited studies have been done, this avenue of research has a promising future. Studies by Larry Mallory and colleagues in the 1990s demonstrated that microorganisms cultured from Hawaiian lava tubes and pools in remote areas of Lechuguilla Cave and Mammoth Cave in Kentucky produced secondary metabolites that could effectively kill cancer cells (L. Mallory, personal communication). An antifungal substance was produced from *Bacillus licheniformis* A12, which was isolated from a cave (Gálvez *et al.*, 1993). *Streptomyces tendae*, isolated from Grotta dei Cervi in Italy, produced a novel antibiotic complex, cervimycin, that may help fight multidrug-resistant pathogens (Herold *et al.*, 2005). The activity of the antibiotic altramiramycin, from a bacterium isolated from Altramira Cave in Spain, has also been tested (Anonymous, 2003). Some investigators of moonmilk speculate that this substance was applied to wounds so that patients could benefit from moonmilk's putative antimicrobial properties (Shaw, 1997). Because limited studies have been done, this avenue of research has a promising future.

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Ecosystem Science and Karst Systems

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INTRODUCTION

Ecosystem science focuses on two major functional attributes of ecological systems: energy flux and nutrient biogeochemistry. Historically, the study of these ecological functions in karst has lagged study in other fields such as biodiversity, evolution, and hydrology. This lag is not due to the insignificance of ecosystem functions in karst, rather it is most likely a result of the relative youth of the field of ecosystem ecology, which did not begin to fully emerge until 1960's and 70's. However, the pace of advances in ecosystem science in karst has not matched that focused on other ecosystems on the surface. This is ironic considering that perhaps the first and best known example of ecosystem ecology was done in a karst ecosystem; that is the development of an energy budget for Silver Spring by Eugene Odum (1957).

Why study ecosystem function in karst? First, energy availability is considered a key driver of the ecology and evolution of cave communities. Caves have long been thought to be energy-limited because of the absence of photosynthesis and the features of cave animals such as reduced metabolic rate, larger but fewer eggs, and increased longevity are considered to support this assertion (see review by Hűppop, 2000). Second, karst ecosystems have much to offer to the study of ecosystem science in general. Some karst ecosystems are exclusively heterotrophic while others are fueled by internal chemolithoautotrophy and both represent endpoints in the broad spectrum of ecosystem types. The study of extremes often yields insights into the more common. Third, future changes related to human activity such as global climate change and intensifying human landuse are likely to be manifest in karst through alterations to ecosystem function. For example, changes to surface vegetation and hydrology, both likely outcomes of climate and landuse change, will undoubtedly influence energy flux in karst.

What is the current state of ecosystem science in subterranean habitats? In this brief review, I consider:

- Appropriate scales for examining ecosystem function
- Foodweb structure and energy sources
- Spatial and temporal variation in ecosystem function
- Energy limitation in karst
- Nutrient biogeochemistry

APPROPRIATE SPATIAL SCALES FOR EXAMINING ECOSYSTEM FUNCTION

The field of ecosystem ecology was revolutionized by linking abiotic and biotic systems at large scales (i.e. an ecosystem). The classic example is the use of a watershed as a unit of study (Bormann and Likens, 1967). This approach allowed for the construction of input/output budgets and subsequent large-scale experimental work. Raymond Rouch and his colleagues pioneered the idea of using a karst basin as an ecosystem, akin to the use of a watershed on the surface. In a series of more than 20 papers (summarized in Rouch, 1986) Rouch measured the inputs and outputs of animals by sampling springs draining different portions of the Baget Basin. Rouch also integrated geological and hydrological thinking by examining subcomponents of the basin, specifically the epikarst, unsaturated, and saturated zones. This integration of the physical sciences into ecology is a hallmark of ecosystem science that has not been fully exploited in karst.

Gibert (1986), in what is the first true ecosystem study in karst, used Rouch's karst basin framework and quantified the flux of organic carbon from springs draining the epikarst and the saturated zones of the Dorvan-Cleyzieu basin in France. Among Gibert's most important findings were that dissolved organic carbon represented a larger flux than particulate organic carbon, that those fluxes were spatially and temporally variable, and that microbes were likely to be key players in mediating energy transfer between organic carbon and animals in karst. The use of a karst basin as an ecosystem is an important approach, but quantifying ecosystem processes at that scale is made difficult by the lack of accessibility to much of karst basins (e.g. the epikarst). This is one reason that we have seen little progress beyond that of Rouch and Gibert on studying ecosystem function at the basin scale.

The decade following the work of Gibert was relatively uneventful, until new work emerged that focused on a smaller spatial scale, the stream reach, which integrated the thinking and methods of stream ecology into the study of karst (e.g. Graening and Brown, 2003, Simon *et al*., 2003). Work in this area has expanded our knowledge of food web structure, energy sources, and organic matter dynamics in the unsaturated zone of karst. This finer-scale approach has not yet been applied to the epikarst and saturated zones of aquifers, probably because of the difficulty in accessing and sampling these subsystems. Virtually all of the research done at any spatial scale in karst has been descriptive or comparative. To date, no ecosystem-scale experimental manipulations, such as the catchment logging at Hubbard Brook (Bormann and Likens, 1967), have been attempted. At the reach scale, only a few small-scale manipulations of organic matter or nutrients have been attempted. (e.g. Simon and Benfield, 2001). This lack of an experimental approach is perhaps the most glaring weakness of the work done on ecosystem function in karst.

FOOD-WEB STRUCTURE AND ENERGY SOURCES

We now have a much better picture of what karst food webs look like and a better understanding of what organic matter sources fuel these food webs than we did only a decade ago (Fig. 1). This advance is largely due to the use of stable isotopes. The natural abundances of stable isotopes, especially ^{13}C and ^{15}N, are now widely used tools for untangling food-web structure and sorting out the important energy sources to foodwebs. This approach has been used to delineate a chemolithoautotroph-based foodweb (Sarbu *et al*., 1996) and the detritus-based foodwebs of 4 cave streams (Graening and Brown, 2003; Simon *et al*., 2003). Other, partial food chains also have been elucidated using stable isotopes (e.g. Opsahl and Chanton, 2006). The chemolithoautotroph-based foodweb, both terrestrial and aquatic, comprised 3 trophic levels including bacteria, invertebrate grazers and predators.

The 4 detritus-based cave streams were also comprised of 3 trophic levels: detritus and associated microbes, invertebrate grazers and invertebrate and vertebrate predators. There appears to be no quantitative data about food web structure or energy use for the terrestrial foodweb in detritus-based karst systems. Beyond trophic levels, there remains considerable ambiguity in the specific trophic interactions among species. In fact, we know surprisingly little about what individual taxa can and do eat in caves. Stable isotope tracers (e.g. Simon *et al*., 2003), analyses of gut contents, and feeding trials (e.g. Simon and Buikema, 1997) should provide further resolution of trophic interactions in karst food webs.

The primary energy sources to most aquatic communities in karst are dissolved and particulate organic matter. While dissolved organic matter has been recognized as a potential food (Gibert, 1986), particulate matter such as leaves, wood and guano has typically been thought of as very important foods in “food-poor” caves. Somewhat surprisingly, the stable isotope data for the four detritus-based cave streams all suggested that leaves, wood and bat guano were largely unused by most cave animals (Graening and Brown, 2003; Simon *et al*., 2003). Rather, microbial films on rocks (epilithon) and fine sediments were much more important foods for all animals. An experimental addition of a stable isotope tracer that labeled microbial films further confirmed that energy fixed in microbial biomass was incorporated in grazers and, ultimately predators (Simon *et al*., 2003). The most likely source of energy fueling those microbial films, based on natural abundance of ^{15}N, was dissolved organic matter originating from soils and percolating through the epikarst. Why cave animals rely mostly on microbial films and dissolved organic matter may be related to the relative reliability of those foods compared to leaves and wood which are patchily distributed, but the true answer is unknown. One caveat to the results of these studies is that they represent snapshots in time and indicate only foods that were important over perhaps the previous months. Energy sources such as leaves and wood may be quite important in brief, periodic pulses and/or in restricted locations in basins.

SPATIAL AND TEMPORAL VARIATION IN ECOSYSTEM FUNCTION

Ecosystem function is likely to vary considerably over space and time considering the heterogeneity of aquifer structure and temporal variability in hydrology. Both of these factors likely contribute to variation in the availability of organic matter and physical conditions (water velocity, temperature) that can influence ecosystem function. Within karst basins, organic matter input and availability is both spatially and temporally variable. Gibert (1986) and Simon *et al.* (2001) found that concentrations of dissolved organic carbon were higher in the saturated zone than in the epikarst of the Dorvan basin in France. The amounts of organic matter present in streams varies within aquifers (Simon and Benfield, 2001) and is linked, in part, to the presence of openings to the surface that permit entry of coarse organic matter such as leaves and wood. There can also be substantial spatial variation in decomposition rates. In Organ Cave, USA, for example, variation in leaf breakdown rates among cave streams spans the range of breakdown rates observed among surface streams (Simon and Benfield, 2001). That spatial variation was largely explained by differences in the invertebrate communities, particularly the abundance of *Gammarus minus*, a leaf-shredding amphipod that is a stygophile. The distribu-

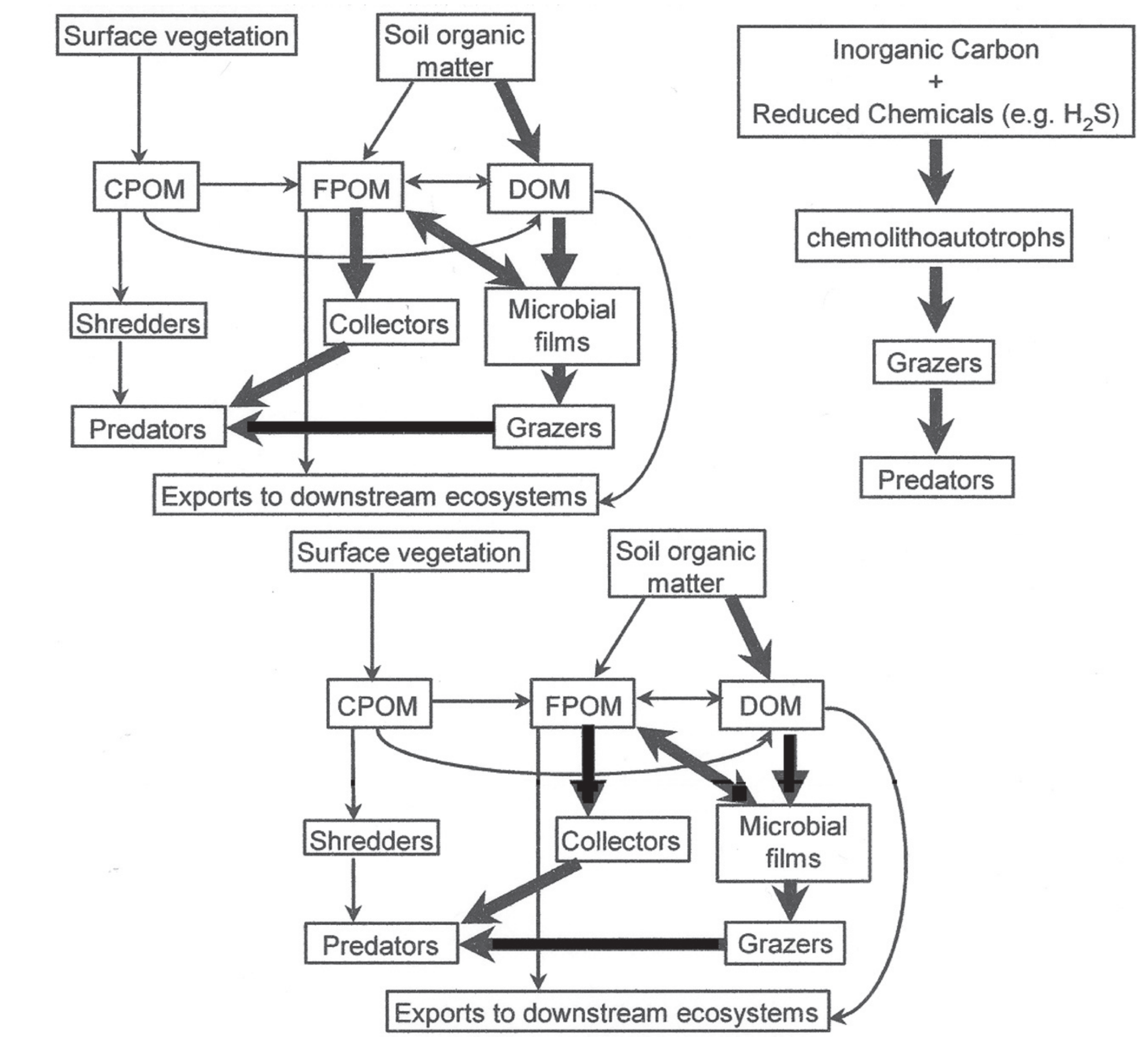

Figure 1. Generalized aquatic foodwebs in karst. Arrows indicate energy flux and arrow size indicates presumed importance of each flux based on existing data. CPOM = coarse particulate organic matter, FPOM = fine particulate organic matter, DOM = dissolved organic matter.

tion of *G. minus* in Organ Cave, and therefore the spatial pattern of leaf breakdown, apparently is the result of the historical changes in aquifer structure that influenced *G. minus* invasion into the cave (Culver *et al.*, 1995). Aside from these few examples, we have little understanding of the extent of spatial variation in energy or nutrient input or what controls that spatial variation. Such spatial variability may be quite important in regulating community structure. For example, at a broad geographic scale, subterranean biodiversity corresponds to surface productivity (Culver *et al.*, 2006). Whether there are "hot spots" or "hot times" of energy input and processing that drive community diversity within karst basins is unknown.

Temporal variation in organic matter and nutrient availability can be considerable in karst and is strongly influenced by variability in hydrologic inputs. Concentrations of organic carbon, nitrate and microbial concentration in the water column all respond to flood pulses. In some cases concentrations of DOC and bacteria are positively correlated with discharge, but not necessarily in all locations within a basin (Pronk *et al.*, 2006, Simon *et al.*, 2001). Many parameters demonstrate a clear temporal lag in their relation to flood pulses. For example, Pronk *et al.* (2006) found peaks in bacterial density and concentrations of dissolved organic carbon and nitrate that lagged peaks in discharge by several days in a Swiss aquifer. These lags in response are probably due at least in part to differences in travel times of water through the various flowpaths through soils and the aquifer. Linking hydrological information to measures of organic matter and nutrient availability (e.g. Pronk *et al.*, 2006) will be critical for developing conceptual and predictive models of both spatial and temporal variation in ecosystem function in karst.

ENERGY LIMITATION IN KARST

The idea that karst foodwebs are energy limited is firmly entrenched despite the lack of any experimental evidence. Anecdotal and indirect evidence both support and refute the idea of energy limitation. Anecdotally, cave animals can be quite abundant where organic matter is abundant in caves (e.g. old wooden structures) and where organic pollution occurs (e.g. Simon and Buikema, 1997). Some measures of ecosystem function, particularly stream metabolism and leaf decomposition, shed some light on the topic. Compared to surface streams, the amount of organic matter in cave streams is low (Simon and Benfield, 2002), except where organic pollution occurs (e.g. Graening and Brown, 2003). Despite low abundance of organic matter, the rate of cave stream respiration can be disproportionately high, resulting in high specific respiration and rapid organic matter turnover times (Simon and Benfield, 2002). This efficient and rapid use of organic matter is consistent with energy limitation. Rates of leaf breakdown have been measured in at least four different studies (Brussock *et al.*, 1988; Brown and Schram, 1982; Galas *et al.*, 1996; Simon and Benfield, 2001). If energy is highly limiting, one would expect leaves to be rapidly consumed and leaf breakdown rates to be high. However, in most of the caves studied, leaf breakdown was quite slow relative to surface streams, possibly related to an observed lack of animals that could consume, or "shred", the leaves that enter caves. In other experimental results, higher leaf breakdown rates were observed in cave streams. In Organ Cave, USA, leaf breakdown rates were very rapid, but only where *Gammarus minus*, a stygophile, was abundant. It may be the case that energy limitation varies within and among karst basins. The issue of energy limitation will remain unresolved until energy availability is experimentally manipulated in a karst system.

NUTRIENT BIOGEOCHEMISTRY

Compared to studies of organic matter, less is known about biogeochemical function in karst. The interactions of microbes in geochemistry, for example cave formations, has received much recent attention and our understanding of chemolithoautrophic ecosystems is rapidly expanding (see the report by Summers-Engel and Northup). However, there are very few measures of nutrient (e.g. N, P, S) uptake, retention, transport and transformation in karst. In many karst systems the availability of N and P may be relatively unimportant because they are generally abundant. For example, in Organ Cave, USA ammonium uptake in streams was relatively low compared to surface systems and the availability of N and P did not limit microbial biomass or respiration on wood substrates (Simon and Benfield, 2002). In this case, the concept of nutrient spiraling was a useful tool for studying biogeochemistry in the stream flow component of a karst aquifer. Different techniques and conceptual models will be needed for other subsystems such as the epikarst and saturated zones in karst. In chemolithoautotrophic systems, the availability of inorganic elements (especially C, N, S and Fe) may be critical to limitation of biological productivity.

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The Struggle to Measure Subterranean Biodiversity

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INTRODUCTION

In spite of the considerable interest in caves as ecological, evolutionary, and microbiological laboratories (see Poulson and White, 1969; Culver *et al.*, 1994; Engel *et al.*, 2004), the heart of speleobiology remains the description and explanation of species diversity. While the study of biodiversity is important in any habitat, it seems especially so for subterranean habitats. There are undoubtedly several reasons for this but certainly the often bizarre morphology of subterranean animals combined with the seemingly inexhaustible supply of undescribed species is a major component.

What is the current state of biodiversity studies in subterranean habitats? In this brief review, I will take a broad view of subterranean biodiversity, and consider

- Species description and identification
- Sampling strategies and completeness
- Biodiversity mapping
- Explanations of biodiversity patterns

For the first topic (species description and identification), I will focus on the situation in the United States, but for the others I will take a more international perspective. An international perspective is not only appropriate but essential because many of the recent advances come from outside the U.S., especially Europe.

If this review had been written ten years ago, there would have been very little to say about the last two points, and not that much to say about sampling completeness. In the last ten years there has been a remarkable increase in quantitative approaches to sampling and biodiversity mapping. This has been the result of many factors including the usual increases in computer capacity and capability. There are two other key factors worth noting. The first is the emerging generation of speleobiologists with strong quantitative skills and a focus on subterranean biodiversity. Among the most notable of these are Stefan Eberhard (Australia), David Ferreira (France), Tristan Lefébure (France), Tanja Pipan (Slovenia), Katie Schneider (U.S.A.) and Maja Zagmajster (Slovenia). The second key factor was the multinational study of European aquatic subterranean biodiversity, **P**rotocols for the **A**ssessment and **C**onservation of **A**quatic **L**ife **I**n the **S**ubsurface (**PASCALIS**). A preliminary summary of this work is in Gibert (2005), and a more complete analysis is forthcoming as a special issue of the journal *Freshwater Biology*.

SPECIES DESCRIPTION AND IDENTIFICATION

There appears to be a perception in the United States that species description and identification of cave animals is on the decline. It is certainly true that a remarkable group of taxonomists, including Barr, Bowman, Christiansen, Ferguson, Hobbs Jr., Hoffman, Holsinger, and Muchmore, taxonomists who largely defined the field in the U.S., are deceased, retired, or close to retirement. Nevertheless, species description continues at a healthy pace, including monographic revisions (e.g., Zhang and Holsinger, 2003; Miller, 2005) and species descriptions (e.g., Lewis, 2005, Lewis e*t al.*, 2006). This is not to say that there are not groups on which no taxonomist is currently working. These groups include planarians and carabid beetles. It is interesting to note that for both of these groups there have been relatively recent revisions (Barr, 2004; Kenk, 1977), and so this makes them less attractive to the systematists looking for major groups to revise. This is likely to become a growing problem of finding someone to either identify or describe species from relatively well known groups, such as the carabid beetle genus *Pseudanophthalmus*. Novel solutions are needed. Even training many more taxonomists is unlikely to solve this problem since taxonomists tend to work on groups in need of major revision, rather than minor additions.

Another trend has been the internationalization of the taxonomy of subterranean organisms. For example, the Czech scientist Zacharda (1985) and the French scientist Thibaud (1996) are actively interested in American cave mites and Collembola respectively. In addition, the Slovenian scientist Tanja Pipan is collaborating with Janet Reid on the description of cave copepods.

Another intriguing trend is represented by Julian J. Lewis in the U.S., Boris Sket in Slovenia, and in an earlier generation, Claude Delamare Deboutteville in France. They all moved beyond the standard idea that one taxonomist can only specialize on one group to do significant taxonomic work on disparate groups of stygobionts and troglobionts. For example, Lewis has described both isopods and millipedes (e.g., Lewis, 2005; Lewis *et al.*, 2006).

A final trend worth noting is the role (or more accurately the lack of a role) that molecular data plays in the taxonomy of subterranean organisms. There have been numerous studies that have shown that cryptic species are quite common. Among the most striking examples are from the European fauna, summarized by Trontelj *et al.* (2007). They found that all species examined with ranges of more than about 250 km in maximum linear extent actually consisted of two or more cryptic species. One of their examples is the iconic European cave salamander *Proteus anguinus*, which actually comprises at least three cryptic species (see also Gorički and Trontelj, 2006). A good North American example, albeit based on protein rather than DNA variation is that of Kane *et al.* (1992) on the carabid beetle *Neaphaenops tellkampfi*. What is remarkable is that, in spite of overwhelming molecular evidence, none of these cryptic species have been formally described. This would afford taxonomists of subterranean groups the opportunity to set a new standard.

SAMPLING STRATEGIES AND COMPLETENESS

There is probably not even a single relatively species-rich cave, let alone a region for which we can be confident that all cave-limited species (aquatic stygobionts and terrestrial troglobionts) have been discovered and described. In the two most species-rich caves known and among the best studied—Vjetrenica in Bosnia & Hercegovina and the Postojna – Planina Cave System in Slovenia—new species are being discovered (I. Lučič, pers. comm., S. Polak pers. comm.). The most recent issue of the journal *Subterranean Biology* (volume 4) nicely illustrates this point. Among the articles are a description of a 200 km range extension of a stygobiotic isopod in France (Notenboom *et al.*, 2006), a new genus of beetle from Albanian caves (Giachino and Vailati, 2006), and three new species of stygobiotic amphipods from Mediterranean islands (Messouli *et al.*, 2006).

While there is a tendency on the part of some speleobiologists to conclude that no generalizations are possible because sampling is incomplete, this approach is simplistic at best and generally wrong. Of course, we all need to have the best information available, but given that new caves and other karst sites are continually being discovered, it will never be possible to generalize if one waits for completeness. More relevant is how complete sampling is. This may seem to be an impossible question to answer but in fact, there are many techniques available to answer this very question, many of them available in the freeware Estimates (Colwell, 2005).

Two general types of estimates have been used by speleobiologists. The first relies on a species accumulation curve produced by randomizing caves (or quadrats) and selecting 1,2,3… sites at random and re-sampling 100 or more times. For the most part, such accumulation curves have not reached an asymptote (see Schneider and Culver, 2004 for one of the first such studies in caves and Rouch and Danielopol, 1997 for an example from a hyporheic habitat). Exceptions to this have been either very intensive sampling at small scales such as epikarst drips within a cave (Pipan and Culver, 2007) or intensively sampled areas aggregated into equal-sized sampling areas (Culver *et al.*, 2006). The second relies on estimators of the total number of species, typically based on some variant of the ratio of species found in a single site relative to those found in two sites. In several cases, these estimates are on the order of magnitude of 50 percent higher than the number of known species (e.g., Pipan and Culver, 2007).

What remains to be determined is which of these estimators, e.g, Chao2, bootstrap, ICE, etc. are most appropriate for cave data. What makes cave data unique is that unlike most samples in surface environments, the number of species known from a single site does not decline to zero as is typical of samples of surface-dwelling fauna, but rather reaches a nonzero asymptote. This makes biological sense because of the high level of endemism in caves (see Christman *et al.*, 2005). In relatively low diversity cave regions, such as the Walloon karst in Belgium, levels of endemism are lower and the number of species known from a single site does approach zero (Deharveng *et al.*, in press). Whether it makes statistical sense to use these estimators for the cave fauna is still an open question, requiring a more detailed examination of the assumptions used in the various estimators.

There are other approaches to the question of sampling completeness. One idea is adaptive sampling (Christman, 2004), which is a sampling strategy that relies on prior knowledge of where organisms are and modifies sampling accordingly. So, for example, species rich cave areas would be sampled more intensively than species poor areas, which is of course analogous to the frequent claim of sampling bias. It is important to note that species rich cave regions repay additional sampling effort more than species poor regions, a prediction made by Zagmajster (2007) and by Deharveng *et al.* (in press) in a summary of the PASCALIS project. Another approach has been to take historical snapshots of data to determine how much the pattern has changed with time (Culver *et al.*, 2004a; Ferreira, 2005). In general the conclusion has been that additional data have not changed the basic pattern.

All of these statistical and historical analyses assume that sampling at sites is more or less complete and that the sites themselves are a representative sample of subterranean habitats in karst. The work of Meštrov (1962) on superficial subterranean seeps (hypotelminorheic), Gers (1983) on superficial subterranean terrestrial habitats (milieu souterrain superficiel or M.S.S.), expanded the range of subterranean habitats outside karst areas, as did earlier and ongoing work on freshwater interstitial habitats (e.g., Lewis and Holsinger, 1985). More importantly for determining diversity patterns on karst is the work on the fauna of epikarst drips, most extensively studied by Pipan (2005). The rich copepod fauna of epikarst can double the known number of stygobionts in a cave (Pipan and Culver, 2005), especially in North America where subterranean copepods are understudied. Major discoveries of this kind obviously cannot be accounted for by the statistical models of species richness.

In general, the quality of sampling of subterranean biodiversity has seen major advances in the past decade due to several factors. The first of these, and one that is exemplified by the PASCALIS project, is a shift from individual work to collaborative work, involving specialists of many taxonomic groups, an ABTI-like approach. The second is to incorporate more efficient and complete sampling strategies. This includes targeting undersampled regions (like southeast Asia), undersampled habitats (like epikarst), and targeting undersampled groups (like copepods). The third is the internationalization of cave biodiversity studies. This may involve specialists from more and more countries involved in the same projects, and the emergence of new countries and new researchers, such as China and Chinese researchers. Finally, there has been a big increase in the links between biodiversity measurement and conservation issues, and in fact most agencies that fund biodiversity studies require this link.

BIODIVERSITY MAPPING

With the widespread availability of sophisticated mapping software such as ArcMap© and the development of spatial statistical methods such as conditional autoregression (CAR), mapping has become much more informative and predictive. Nearly all investigators who have mapped subterranean species richness have found that it is very heterogeneous, and this creates difficulties on both displaying and interpreting a map. Heterogeneity is greatly reduced if data are grouped into quadrats. This largely eliminates the problem of poorly sampled caves and well sampled caves since the data are lumped together (see Culver *et al.*, 2004a).

The problem of heterogeneity brings about the question of what scale of analysis is optimal, i.e., what is the optimal quadrat size. Zagmajster *et al.* (2008) suggest that the optimal size is when Moran's I (a measure of spatial autocorrelation) reaches its maximum, and apply this to the rich troglobiotic beetle fauna of the Balkans. Zagmajster (2007) has also produced contour maps of species richness based on both observed numbers of species and predicted number based on estimators such as Chao's. This allows an analysis of where it is likely most uncollected species are, and it turns out that in her case, it is in the peaks of richness rather than the valleys.

Another approach is that of Christman (2005) who very elegantly partitioned stygobiotic species richness in counties in the southeastern United States into that predicted by number of caves in the county (local effects), a spatial autocorrelation mesoscale component, and residual variation. It not only displays the pattern of species richness, it also partitions it into local and regional components. Christman also points out the difficult problem of modeling the spatial distribution because of the large number of zeroes, zeroes that may be because no habitats exist in the county, habitats exist but have not been sampled, and habitats exist, have been sampled, but no stygobionts were found. This problem of "zero inflation" is very typical of any large scale mapping of subterranean biodiversity and the problem is far from solved.

Finally, nearly all maps of subterranean biodiversity show not only excess zeroes, but very clear hotspots of species richness, hotspots that we cannot always predict (but see Culver *et al.*, 2004b). This has been observed by Zagmajster *et al.* (2008) for the Dinaric beetle fauna, by Deharveng and Latella (unpublished) for the Chinese beetle fauna, Deharveng *et al.* (2007) for the European stygofauna, and Christman *et al.* (2005) for the single cave terrestrial endemics of eastern North America.

EXPLANATIONS OF BIODIVERSITY PATTERNS

For decades there has been an interest in subterranean biodiversity patterns, initially focused on tropical-temperate comparisons, which were all the rage in ecology especially in the 1970's. The initial pattern of higher diversity in temperate areas was disputed (e.g. Howarth, 1972) but this discussion was framed in the absence of quantitative data. It was not until Deharveng's (2005) summary of species richness in some well-studied tropical caves that it was clear that temperate caves were more diverse, although this has still not been demonstrated quantitatively on a regional scale. A complicating factor is that faunal composition in terms of ecological groups is extremely different in tropical and temperate caves. If guanobionts are included (most are only known from caves), it may change the pattern substantially.

In a quantitative study involving data for more than 1,500 caves, Culver *et al.* (2006) found that troglobiotic diversity in temperate areas was highest in regions of high actual productivity which formed a band in southern Europe and southeastern United States, suggesting that the species richness was controlled by resource availability. On a broad regional scale, others have found other factors to be important, including distance to recent glaciation (Castellarini *et al.*, 2007), hydraulic conductivity (Castellarini *et al.*, 2007), and habitat availability (cave density) (Christman and Culver, 2001). At a smaller scale, chemical factors can be important, as has been shown by Pipan, Blejec, and Brancelj (2006). The multivariate analysis of the causes of differences in subterranean species richness is in its infancy and no clear generalities have yet emerged. Indeed, there is no reason to expect that all cave communities are limited by the same set of factors.

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Evolution in Karst Lineages, Ages, and Adaptation of Cave Faunas

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INTRODUCTION

The study of subterranean faunas has a long and rich history, with the first scientific description of a cave-adapted species occurring in the 1700s. A major theme in biospeleological studies has been the evolution of troglomorphy – the unique suite of traits characterizing 'cave-adapted' species. The suite of changes associated with cave adaptation includes reduction in pigment, eye size, and metabolic rates, and hypertrophy of nonoptic sensory organs and feeding structures. From an evolutionary perspective, the troglomorphic state is interesting for several reasons. First, the highly convergent form can obscure taxonomic relationships among cave-adapted species and among closely related cave and epigean (i.e. surface) species, hindering distributional and diversity studies. Second, because of the universal convergence of form found in the cave environment, being exhibited in structural, functional, and behavioral changes across diverse taxonomic groups, cave-adapted species provide a model evolutionary system for studying the forces that have lead to such a strong convergence and for investigating the relationship between genotypic and phenotypic change.

Molecular techniques employed in the evolutionary study of cave species began in the 1970s with the development of the first major molecular markers, allozymes (protein variants, Avise and Selander, 1972; Carmody *et al.*, 1972; Hetrick and Gooch, 1973; Laing *et al.*, 1976; Sbordoni *et al.*, 1979). As the field of molecular evolution and phylogenetics advanced, the available number of molecular markers increased and the associated analyses became more sophisticated. However, only within the last few years have molecular studies using gene sequences to investigate evolutionary questions in cave faunas become common.

These recent molecular studies in subterranean habitats encompass several major evolutionary themes:

- Biodiversity and phylogeography
- The genetic and developmental mechanisms of cave adaptation
- The evolutionary timeline of cave adaptation

This review will focus on these three general themes, describing the current state of research in each.

BIODIVERSITY AND PHYLOGEOGRAPHY

Since the inception of 'biospeleology' as a scientific field, there has been a strong focus on species descriptions. These taxonomic studies have fueled biogeographic research aimed at understanding subterranean species diversity and distributions. Although these issues have been intensively studied for several hundred years, the application of molecular techniques to appreciating the genetic diversity and biogeography of cave fauna have led to several significant discoveries that have large implications to the way subterranean biodiversity is quantified and protected.

One of the foundations of biogeographic studies is a solid understanding of the distribution of the species of interest. The most striking discovery of recent molecular phylogenetic studies of cave faunas is the identification of cryptic genetic diversity, which impacts our understanding of the distribution of subterranean faunas and their relationships with each other and with epigean species. Specifically, almost every recent, sequence-based molecular study of cave-adapted species has identified at least one cryptic lineage (Buhay and Crandall, 2005; Goricki and Trontelj, 2006; Lefébure *et al.*, 2006b; Buhay *et al.*, 2007), while in some groups cryptic genetic diversity appears to be rampant (Finston *et al.*, 2007; Lefébure *et al.*, 2007). In many cases, this previously unidentified diversity is diagnosed as the presence of cryptic species. Even when morphological-based taxonomy and molecular studies agree on the diagnosis of a cave-adapted species, convergent morphologies often lead to erroneous hypotheses of close evolutionary relationships at higher taxonomic levels (Wiens *et al.*, 2003). For example, molecular studies of the stygobiotic catfish *Prietella phreatophila* Carranza, 1954 and *Prietella lundbergi* Walsh and Gilbert, 1995 indicate that each is more closely related to species from different genera than they are to each other (*P. phreatophila* to *Ictalurus* spp. and *P. lundbergi* to *Ameiurus* spp. (Wilcox *et al.*, 2004). A similar situation exists in cave-adapted *Troglocaris* shrimp species, which represents a non-monophyletic assemblage with several species being most closely related to surface species or different cave-adapted species outside of the genus *Troglocaris* (Zaksek *et al.*, 2007).

This situation calls for the marriage of classic morphology-based taxonomy with molecular techniques to fully understand subterranean biodiversity. One of the best examples of joining these two fields to understand the karst biodiversity is from a study of cave-adapted spider *Cicurina* spp. from Texas, USA. This species complex represents some of the most common problems with taxonomic studies of cave faunas: low population densities and a rarity of encountering individuals, particularly the adult specimens required for accurate species identification and taxonomic description. Using molecular data, immature specimens can be compared to known species for determination of how they fit into the current, morphology-based, taxonomic scheme. In Texas, this approach has led to identification of the federally endangered *Cicurina madla* from more than twice the number of previously reported caves (Paquin and Hedin, 2004). In a broader effort to bridge the gap and provide a way to integrate DNA information with current taxonomic methods, Lefébure *et al.* (2006a) empirically evaluated the correlation between currently described crustacean species and molecular divergence. Based on sequence data for two mitochondrial genes, they propose a species level molecular threshold to be used as a tool for the taxonomic delimitation of new crustacean species.

In the absence of obvious morphological differences due to extreme convergence, molecular phylogenetic studies of cave-adapted species have been successful at diagnosing cryptic genetic diversity and the presence of taxonomic incongruence. At a minimum, these recent studies illustrate a decoupling between molecular and morphological evolution in the cave environment (Lefébure *et al.*, 2006b). If patterns of cryptic genetic diversity within currently described species and misdiagnosed relationships above the species level hold for most subterranean faunas, then taxonomic based studies may be extreme underestimates of subterranean biodiversity and our understanding of basic species-level relationships may be biased (Proudlove and Wood, 2003; Lefébure *et al.*, 2006b; Finston *et al.*, 2007). Although there is considerable debate about how to deal with this cryptic genetic diversity within the existing taxonomic framework, understanding these patterns will greatly impact interpretations of hydrological connectivity patterns, ecological habitat restrictions, geological barriers, climatic and geological vicariant events, and differing invasion scenarios contributing to the genetic structuring of populations (Chippindale *et al.*, 2000; Parra-Olea, 2003; Buhay and Crandall, 2005; Finston *et al.*, 2007).

THE GENETIC AND DEVELOPMENTAL MECHANISMS OF CAVE ADAPTATION

In contrast to their confounding nature in biodiversity studies, the convergent morphologies of the cave-adapted forms provide a unique system for understanding the genetic and developmental mechanisms responsible for constructive (acquired) and regressive (lost) traits. Perhaps the best-studied, cave-adapted species with respect to understanding the issues of convergent trait acquisition and loss is the Mexican cave tetra, *Astyanax mexicanus*. *A. mexicanus* contains both eyed and pigmented surface populations and eyeless, pigmentless cave-adapted populations (Fig. 1), providing an unrivalled system for mechanistic studies of trait evolution. Direct comparisons can be made between a derived trait (the cave form) and its ancestral state (the surface form) within one species. Along with this, the numerous regressive and constructive traits associated with the cave form and the existence of multiple cave-adapted populations arising from independent subterranean colonization events make *A. mexicanus* a model system for research in evolutionary biology (Jeffery, 2001).

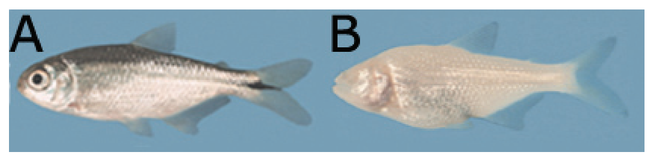

Figure 1. Epigean (A) and hypogean (B) forms of *Astyanax mexicanus* (photos provided by W.R. Jeffery).

Studies of trait evolution in *A. mexicanus* also have a significant history in biospeleology, beginning with classical genetic studies where individuals from different populations were crossed to examine the heritability of eye size and pigmentation (Sadoglu, 1955, 1956, 1958; Wilkens, 1971). More recent genetic studies have used genome-wide linkage maps with quantitative trait analysis to investigate the genetic basis of pigment loss (e.g. the evolution of albinism) in two different populations of cave-adapted *A. mexicanus* (Protas *et al.*, 2006). This study found that albinism in these populations was linked to a known pigmentation gene, *Oca2*. Furthermore, different inactivating mutations were identified, indicating that albinism has evolved independently in the two studied populations by convergent evolution in the same gene (Protas *et al.*, 2006). In one of the few studies to study the genetic basis of cave-adapted trait loss outside of *A. mexicanus*, Leys *et al.* (2005) investigated the eye pigment gene *cinnabar* in independent lineages of subterranean dytiscid water beetles. Similar to the findings of Protas *et al.* (2006), *cinnabar* sequences indicate increased rates of sequence evolution, including mutations leading to the loss of gene function.

Loss of eyes and pigmentation has also been investigated in *A. mexicanus* from a developmental perspective. In particular, developmental studies of eye reduction and loss show a different mechanistic pattern than pigment genes. In cavefish embryos, expanded midline signaling of the *hedgehog* genes inhibits eye formation, leading to lens apoptosis and eye degeneration (Yamamoto *et al.*, 2004). Because *hedgehog* genes may regulate the development of constructive traits in cavefish morphologies such as feeding structures (Jeffery, 2005) and possibly olfactory regions in the brain (Menuet *et al.*, 2007), it has been hypothesized that loss of eyes in *A. mexicanus* is a consequence of the pleiotropic effects of natural selection for constructive traits (Jeffery, 2005). Studies of other cave-adapted traits, such as loss of aggressive behaviors, found at least one *A. mexicanus* population that had not lost aggression (Espinasa *et al.*, 2005). These results indicate that there are differences among traits, and all cave-associated traits need to be examined to determine patterns and mechanisms of acquisition and loss.

In the history of biospeleological research, there has been a long-standing debate over whether the convergent loss of traits in the cave-adapted form is due to selective pressures or neutral mutations and drift (Kane and Richardson, 1985). However, the most recent genetic and developmental studies highlighted here make it clear that this debate is no longer valid. Instead, these studies emphasize that both selection and drift play a role in cave-adapted morphologies; at what point along the evolutionary path to cave adaptation selection and drift act, and the degree to which each is the driving force, depends on the trait. Quantitative trait loci (QTL) mapping in *A. mexicanus* illustrate the differences among cave-associated regressed traits. Using this approach, Protas *et al.* (2007) showed that cave alleles at eye or lens QTLs caused size reductions, consistent with evolution by natural selection while QTLs associated with melanophores caused both increases and decreases in number, consistent with drift. The emerging theme is that trait loss, or regression, can be the result of different evolutionary forces and genetic and developmental pathways. Some genes appear to be predisposed to be targets for evolutionary forces effecting morphological change (Protas *et al.*, 2007), such as the pigmentation genes *Oca2* and *cinnabar*. This may be due to a lack of deleterious pleiotropic connections and gene structure, leading to similar morphologies evolving by independent mutations or changes in expression patterns in the same genes. It remains to be seen whether the patterns of evolutionary change highlighted in the studies presented here for just a few cave-adapted species hold across other regressive traits within *A. mexicanus* and within other cave-adapted species.

THE EVOLUTIONARY TIMELINE OF CAVE ADAPTATION

An important aspect of understanding troglomorphy is the evolutionary time required to achieve the cave form. Because it is difficult to pinpoint the time of subterranean colonization and isolation from the surface, the time of cave adaptation is generally thought of in relative terms, where the degree of eye and pigment reduction indicates the period of cavernicolous evolution and therefore the relative phylogenetic age of each species (Aden, 2005). Advances in molecular methods, however, allow for the estimation of divergence times among subterranean lineages. Estimations of divergence times in cave species have been used most often to correlate the time of lineage splitting with geologic or climatic events that may have caused subterranean invasion, such as Pleistocene glaciations or late Miocene aridification. With one exception where pairs of cave-adapted Orconectes spp. have estimated divergence times ranging from 102-125 Ma (Buhay and Crandall, 2005; but see Trontelj, 2007 for disagreement with these ages), most estimates of subterranean colonization times are younger than ~16 Ma. Strikingly, these estimates fall within similar ranges, even through the estimates come from different cave-adapted taxonomic groups and regions of the world (Australian dytiscid beetles, 9.0-15.8 Ma - Cooper *et al*., 2002; Dinaric karst isopods, 0.8-3.9 Ma - Verovnik *et al.*, 2004; European amphipods, >13 Ma - Lefébure *et al.*, 2006b; Australian crangonyctoid amphipods, 4.1-14.7 Ma - Cooper *et al.*, 2007; Mexican cave tetras, 0.3-5.2 Ma - Porter *et al.*, 2007; Dinaric karst decapods, 3.7-5.3 Ma - Zaksek *et al.*, 2007).

In the efforts to understand how fast (or slow) acquisition of the cave morphology occurs, *A. mexicanus* again offers a unique model. The primary mode of *A. mexicanus* subterranean colonization is via stream capture, with most of the captured surface drainages no longer supporting epigean populations (Mitchell *et al*., 1977). Because stream capture events provide a time frame for the identification of discrete colonization events by *A. mexicanus,* divergence times of lineage splits between cave populations and their most closely related surface populations can be linked directly to the time of subterranean evolution. Although it is not reasonable to assume that all of the traits evolve at the same time, based on hydrogeologic constraints, fossil calibrations, and cyt*b* sequences data, acquisition of the troglomorphic form in A. *mexicanus* is estimated to be younger than 2.2-5.2 Ma (Porter *et al.*, 2007). It should be noted that these times correlate to the time of subterranean colonization, not necessarily to the time of cave-associated trait acquisition. However, estimating lineage splitting in *A. mexicanus* does place an upper limit on the time required for the acquisition of the cave form, providing a working hypothesis for future studies of different species.

CONCLUDING REMARKS

The application of molecular tools to the study of subterranean systems has led to new insights about the evolution of cave species and about the evolution of traits in general. It is clear from the studies that have been highlighted here that to fully understand subterranean biodiversity a union of molecular data on cryptic genetic diversity with classic taxonomy is required; however, the methods needed to accomplish this are not yet well developed. Although much has been learned about the evolution of trait loss from the *A. mexicanus* system, the interplay between trait loss and acquisition is also not well understood. As the evolution of the cave form is investigated in additional species, it will be fascinating to determine if the same mechanisms and forces as observed in *A. mexicanus* play a role in explaining the extreme convergence of cave adaptation. Cave-adapted species remain a model system for studying basic evolutionary processes and the molecular-based studies highlighted here illustrate that there is still much to be learned.

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Karst Resources and Other Applied Issues

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INTRODUCTION

Applied issues in karst span a wide range of topics, including land-use planning and impacts, availability of water resources, flooding, subsidence, building stability, and degradation of water quality. The importance of these issues cannot be overstated given the large number of people that reside in karst regions and ecosystems that rely on karst environments and water to sustain life. Furthermore, we need to conserve karst systems for use in studying large scientific questions and fundamental processes.

Most of the work on applied issues has been focused on case studies and solving specific problems: for example, remediating sinkholes and subsidence for engineering purposes, decreasing flood risks, and tracking aquifer contaminants. The site-specific nature of an applied approach provides important local solutions, but it does not always address the fundamental scientific questions or mechanisms that can allow the answer to be generalized to other systems.

Many of the issues related to land and water resources overlap with research being conducted in non-karst areas. However, karst systems have some unique characteristics that require directed attention and research:

- Close connections between surface and subsurface processes render karst systems highly vulnerable to impacts from surface activities.
- Spatial heterogeneity hinders our ability to easily monitor and assess water quality and quantity.
- The rates of physical processes are highly variable.
- Subsidence may occur at an almost imperceptible rate or be nearly instantaneous. Contaminants may be rapidly flushed through the system or trapped indefinitely. Water flow at springs may be consistent through time or change rapidly in response to storm events.

Although karst systems have some unique characteristics, these topics provide insight to other heterogeneous sites such as fractured rock and urban systems with pipe infrastructure. Management for sustainable-resource use requires that we better understand the fundamental processes in karst systems so that we can better translate between them and better predict likely impacts.

The applied issues are divided into three categories by type: (1) water quality, (2) water quantity, and (3) geotechnical issues. The relevance and implication related to land use at defense installations are also discussed.

WATER QUALITY

Water quality can be degraded by the transport of suspended sediments or the introduction and transport of chemical contaminants and biological agents. These can be input into the system either quickly via sinking streams, sinkholes, and enhanced vertical pathways or slowly via a more dispersed route (Figure 1). Spatial distribution ranges from individual point-sources such as spills and underground tanks to non-point sources such as widespread agricultural spraying. Once in the aquifer, contaminant fate and transport is dependent on the type of contaminant and its chemical properties, as well as the chemical and physical properties of the aquifer. Transport of contaminants can range from rapid movement (velocity of meters per second) through the system and discharge at springs, to trapping mechanisms that result in long-term storage (velocity of less than a meter per year) with little or only very gradual release. Continuous monitoring can help capture these highly variable release mechanisms. New model development needs to incorporate functions to better interpret these processes and the varied contaminants that may be present in karst.

Sediment Transport

Numerous recent studies have focused on the presence and transport of sediments in karst systems. How and when sediments move are closely linked to water quality from both a turbidity and contaminant transport standpoint. Many types of contaminants associate with solids and may be either stored or transported along with the sediments. The current direction of the research is looking at storage of sediments and larger materials within the system, suspended sediment flux during storms,

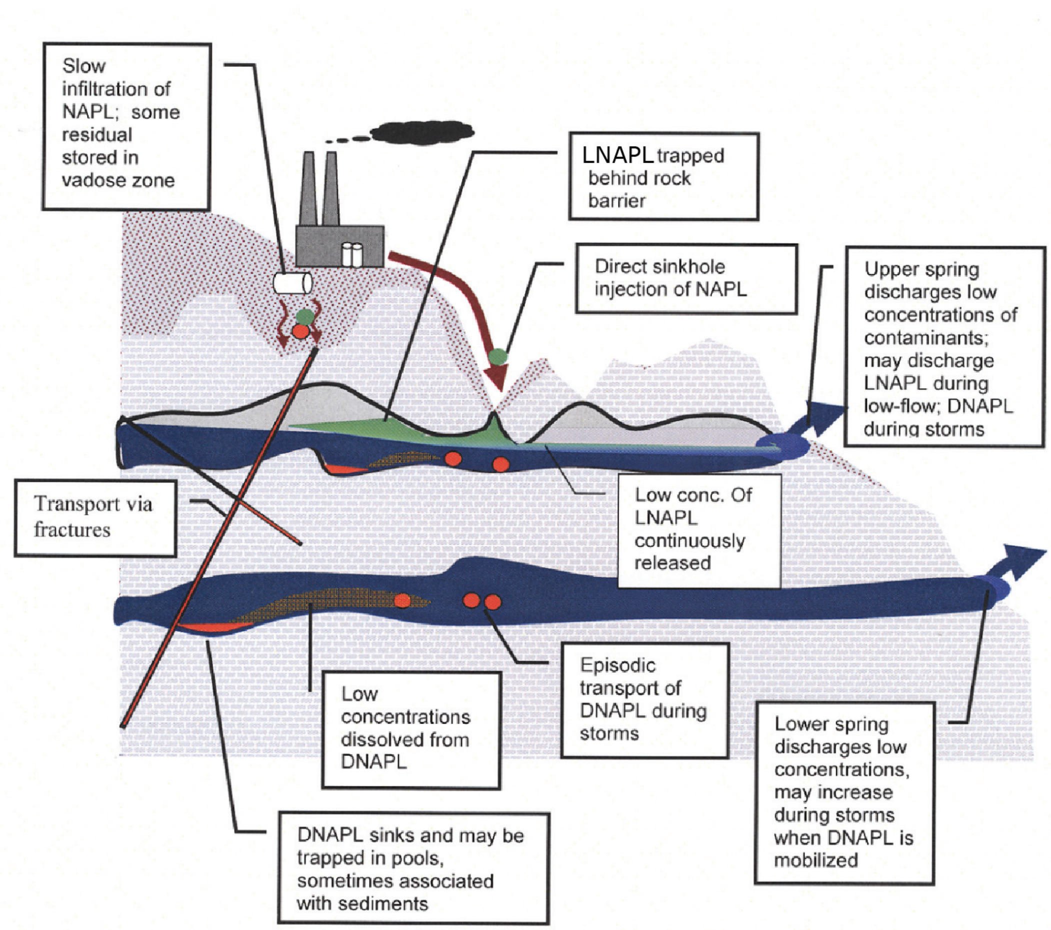

Figure 1. Karst trapping mechanisms and transport styles for light and dense non-aqueous phase liquids (LNAPL and DNAPL, respectively).

the nature of the transported sediments, and mathematical strategies for comparing sediment transport to other parameters.

Transport is typically linked to turbulent flow, and thus to storm events. Both particle size (Atteia and Kozel, 1997) and mineralogy (Mahler and Lynch, 1999) can change over the course of a storm. Studies have identified sediments transported from the surface during storms (Ryan and Meiman, 1996) and due to re-suspension of sediments already in the system (Herman *et al.*, 2008; Marshall *et al.*, 1998; Pronk *et al.*, 2006).

The relationship between discharge and turbidity varies dependent on antecedent conditions (Toran *et al.*, 2006). Threshold discharge values may exist for sediment transport and re-mobilization of larger materials already within the system (White, 1988). A threshold-crossing event was recently observed at Pennsylvania spring, following three major hurricanes, when the sediment discharged at a spring increased by nearly an order-of-magnitude above previous records (Herman *et al.*, 2008).

Autocorrelation and spectral analysis are being used to determine the relationship between turbidity and discharge (Bouchaou *et al.*, 2002; Padilla and Pulido-Bosch, 1995). Time series analysis can help identify different time components in the data and residence time of the input-pulse (a storm) through the system.

Transport of particles is poorly explained through the use of traditional dissolved tracers. Therefore, the design and testing of solid-phase tracers transported in suspension is an active area of research. Success has been demonstrated for lanthanide-labeled clay (Mahler *et al.*, 1998; Ting, 2005), DNA-labeled clay (Mahler *et al.*, 1998), bacteria (Auckenthaler *et al.*, 2002; Dussart-Baptista *et al.*, 2003), europium-tagged bacteria (Ting, 2005), and microspheres (Auckenthaler *et al.*, 2002). The lanthanide-labeled clay tracer (Mahler *et al.*, 1998) was transported faster than the co-injected dissolved solute (NaCl for surface water, rhodamine for karst ground water). Other researchers have observed similar results suggesting this may be due to matrix exclusion of particles injected into the soil zone

(Auckenthaler *et al.*, 2002), although this does not explain the surface water results for the lanthanide-labeled clays. These studies argue strongly for the need for coupled tracers to clarify different modes of transport.

Inorganic Contaminants

Inorganic contaminants are among the most intensively-studied of the contaminant groups. Some inorganic compounds, such as nitrates and chlorides, are highly water soluble. Others, such as heavy metals, are more typically associated with solids either through precipitation or sorption.

Nitrate and nutrients have widespread sources in agriculture, industry, and wastewater. Nitrate has been shown to vary in concentration over several time scales. Storm-based studies have shown that nitrate can either increase or decrease during storms, depending on the source type. Nitrate associated with surface contamination, such as feed lots, increases at springs during storm events as the overland flow injects the contaminant into the aquifer. If the nitrate is stored within the system and continuously released, its concentration can decrease during storms. Nitrate can also change seasonally with land application of fertilizers (Panno and Kelly, 2004). Isotopic studies of the $\delta\ ^{15}N$ and $\delta\ ^{18}O$ in the nitrate ion link contamination to fertilizers that have undergone some denitrification (Panno *et al.*, 2001). Strategies to predict nitrate concentrations in springs have included multi-regression models (Peterson *et al.*, 2000), autocorrelation analysis (Jones and Smart, 2005), and tributary-mixing models (Perrin *et al.*, 2006). All of the approaches produced results that were site-specific, suggesting that the flow-nitrate concentration relationship is not readily generalized.

Another effluent and agriculturally-related contaminant receiving recent attention is phosphorous which has been studied in Irish springs (Kilroy and Coxon, 2005). Phosphorous was found to increase during storm events and travel primarily in the particulate form – indicating its transport might be closely associated with suspended sediment movement.

Chloride and sodium are highly soluble ions often attributed to urban and road salt sources. These concentrations are often highest in the springtime (Panno *et al.*, 2001). Retardation of sodium relative to chloride, in a road-salt contaminated fractured aquifer, has been attributed to temporary sequestration via ion exchange (Werner and diPretoro, 2006).

Heavy metals and metalloids typically associate with solids and are transported in conjunction with suspended sediments (Vesper and White, 2003, 2004). The neutral to alkaline pH of karst settings renders most metals insoluble via either precipitation or sorption. Elevated metal concentrations have been found in urban spring sediments (Gutiérrez *et al.*, 2004) and springs downgradient from metal contaminant sources (Vesper and White, 2004).

Microbes and Pathogens

Microbes are readily transported through karst aquifers. They have been shown to enter the aquifer from surface sources (Marshall *et al.*, 1998; Ryan and Meiman, 1996) and from pumping-induced surface-water infiltration (Mahler *et al.*, 2000). Re-suspension of fecal coliform already in the system has also been suggested (Davis *et al.*, 2005; Marshall *et al.*, 1998). Inoculated chambers placed in karst springs and streams showed that *Excherichia coli* can survive up to four months after introduction into the system (Davis *et al.*, 2005).

Although coliform concentrations have been shown to increase during storms (Ryan and Meiman, 1996), *Crytosporidium parvum* oocysts have been found in spring water during baseflow conditions (Kuczynska *et al.*, 2003). Several studies have indicated that although microbial concentrations in springs are generally high during storms when the turbidity increases, bacteria can also be present during baseflow or low-turbidity conditions (Dussart-Baptista *et al.*, 2003; Pronk *et al.*, 2006). A comparison of sessile (attached) and planktonic (non-attached) bacteria transport from a sinkhole to a spring found that the planktonic bacteria were more closely correlated to turbidity than the sessile bacteria (Dussart-Baptista *et al.*, 2003).

Anthropogenic Organic Compounds

Organic compounds have a wide range of chemical characteristics and may preferentially partition into numerous physiochemical compartments: gases, dissolved in water, incorporated into solids, sorbed to organic or inorganic solids, in biota, or as free-phase product. This in turn controls their transport and storage. The distribution can be estimated using the chemical properties of the compound (vapor pressure, solubility, acid-base dissociation) and partitioning coefficients (Henry's law constant defines the relative concentrations between air and water, octanol-water portioning coefficients as proxy for mass transfer between organic phases and water, various solid-water partitioning coefficients).

Knowledge of the environmental setting is critical. For example, solubility is a function of temperature, solution ionic strength, and the presence of co-solutes; sorption to solids is closely tied to the fraction of organic carbon present. In addition to the transfer of contaminants between physiochemical or system compartments, transformation reactions can occur. If contaminants are destroyed (degraded), product compounds can be created which are also hazardous.

Studies focused on the presence and transport of organic compounds in karst settings are increasing. In additional to the temporal variation found for all water quality parameters, low-solubility organic compounds can be present as non-aqueous phase liquids (NAPLs).

Pesticide concentrations in springs vary seasonally with land application (Panno and Kelly, 2004; Pasquarell and Boyer, 1996). The presence of atrazine on suspended sediments indicates that pesticides may be stored in aquifer sediments and transported periodically (Panno and Kelly, 2004). The storage and breakdown of atrazine in the soil zone was tracked seasonally by the ratio of atrazine to its degradation–product desethylatrazine (DES) (Pasquarell and Boyer, 1996). PCBs were studied at a spring in central Indiana and found to increase in concentration during storms (Krothe and Fei, 1999). Natural estrogen, a tracer for waste products, has been measured in the form of 17 β-estrodial in eight Missouri springs (Wicks *et al.*, 2004) and five Arkansas springs (Peterson *et al.*, 2000). Periodic samples from Meramec Spring in Missouri indicate that the concentration increases with discharge.

NAPL contamination can exist either as residual (held to grains via surface tension) or pooled (Fig. 1). Both forms act as long-term sources, slowly releasing contaminants into water. Light NAPLs (LNAPL) are less dense than water and float on the top of the water table. They may be trapped or decanted in a karst aquifer due to the physical geometry of the flow system (Ewers *et al.*, 1992) (Fig. 1). At Fort Campbell Army Airfield, a 16-foot thick layer of jet fuel was documented, although no free product was present in Quarles Spring – the traced output from the airfield (Ewers *et al.*, 1992). Later studies of Quarles Spring indicated the presence of a "well washed jet fuel" associated with the spring sediments with only low concentrations of volatile organic compounds (VOCs) in the water (Vesper and White, 2006). Ewers and others also investigated the transport of LNAPL at a site in Richmond, Kentucky. A leaky underground storage tank was traced to two springs using dyes; however, only one of the springs contained detectable VOCs (Ewers *et al.*, 1992).

Dense NAPL (DNAPL) sinks during transport, resulting in a flowpath that may not match the overall direction of ground water flow (Fig. 1). Crawford and Ulmer tracked a 1990 spill of chloroform and styrene from a train derailment to Wilson Spring, Tennessee (Crawford and Ulmer, 1994). Wells installed in the aquifer identified DNAPL chloroform and LNAPL styrene near the spill. The DNAPL migrated down dip along a confining layer rather than in the direction of ground water flow. Wilson Spring discharged low levels of both compounds and, after large storms, styrene product. A more recent study of springs contaminated by VOCs (Williams and Farmer, 2003; Williams *et al.*, 2006) found that Wilson Spring continues to discharge chloroform and styrene. Chloroform concentrations increase during storms, with the greatest change after first-flush storms. At Cascade Spring, Tennessee, contaminated with cis-1,2-dichloroethene (Williams and Farmer, 2003; Williams *et al.*, 2006), the VOC concentration was observed to decrease during storms.

Overarching Water Quality Issues Unique to Karst Systems

Several issues exist for all types of water-quality parameters and are unique to karst systems:

- Source location and mode of introduction often controls patterns of contamination. It is possible for contaminant injection via sinkholes or exposed epikarst during storm events. This process can rapidly introduce mobile sediments, soluble contaminants, particulate and solid-associated contaminants, and microbial pathogens. Flow systems of this type can result in highly variable contaminant concentrations at downgradient locations. Infiltration through a thick soil cover can also lead to long-term sources that are mobilized episodically.
- Temporal variability is a common characteristic of contaminant transport in karst. Whereas some contaminant concentrations may increase during storms, others have been shown to decrease. The direction of change may depend on the contaminant properties and where it is stored within in the system. Well-defined contaminant plumes are uncommon; episodic transport is the norm.
- Karst systems have NAPL trapping mechanisms not typically found in other types of aquifers. These can cause storage and retardation of contaminants and hinder remediation attempts.

WATER QUANTITY

Knowing the sustainable yield for a karst aquifer, and how climate and land-use activities impact that yield, is essential information for water-resource management. Unfortunately, many of the techniques used to determine storage and sustainable yield in porous media aquifers (e.g., pump tests) are not directly applicable to karst aquifers or other highly heterogeneous systems. Recent attempts at evaluating aquifer yield have employed water balances, spring flow and hydrograph data, and age-dating to determine the residence time of water underground.

Detailed water balances have been determined in some places. A water balance was determined for the Trnovsko-Banjška Planota Plateau in Slovenia (Trisic, 1997). Spring flow data and hydrograph separation has been used to estimate baseflow and storage in karst aquifers. However, spring discharge may be limited due to conduit size, connections between basins during high flow, delivery to intermittent springs, and dynamic epikarstic storage (Bonacci, 2001). Land use and engineering change can cause significant decrease in sustainable water yield. Storage losses have been witnessed in China due to deforestation in the upland areas of the aquifers in the Stone Forest (Huntoon, 1992). Without the flow-buffering capacity of storage, water resources have become more variable, with increases in both droughts and floods.

Long-term spring monitoring pre- and post-dam building in Herzegovina found that the minimum spring discharge did not decrease but the annual average output did owing to rerouting of recharge water into engineered structures (Milanovic, 2002). Other studies have indicated that urbanization can increase aquifer recharge owing to the increased infiltration from utility infrastructure (Lerner, 2002).

Long-term yield has also been addressed through use of atmospheric tracers used to identify the residence time of water underground or the mixing of old and young waters. Low tritium concentrations during baseflow in Slovenian springs indicate the discharge of storage water greater than 50 years old (Biondic *et al.*, 2006). A suite of tracers, including chlorofluorocarbons, have been used to date the shallow water component in the Floridan aquifer (Plummer *et al.*, 1998; Plummer *et al.*, 1998).

The storage capacity and sustainable yield of a karst aquifer is not readily determined (Bakalowicz, 2005). When karst water supplies are mined or over-exploited, impacts can include salinization, intensification of karst processes, and catastrophic subsidence (Pulido-Bosch, 1999).

GEOTECHNICAL PROBLEMS

Naturally occurring processes such as sinkhole collapse or sinkhole flooding can be accelerated or induced by human activities and have created millions of dollars in losses and a number of fatalities. Sinkhole collapse has resulted in the loss of structural support of bridge foundations, roadways, railways, and buildings and has occurred in many states. Ten Multidisciplinary Conferences on Sinkholes & Engineering & Environmental Impacts of Karst have occurred since 1984; the proceedings contain numerous excellent case studies. Failure to take into consideration karst processes in the location and construction of large reservoirs commonly result in either extreme cost overruns in projects and/or the failure of the structures to perform as designed.

Like the other applied issues, most of the research in geotechnical aspects of karst has been focused on local problem mitigation and engineering solutions. Risk analysis and the mapping of hazard areas have been active topics.

Active research topics in applied geotechnical issues include techniques for identifying underground karst features, mechanisms of subsidence and collapse, and stability of dams:

- Various geophysical techniques have been investigated for their use in locating sinkholes and underground structures. Some success has been obtained through use of resitivity and microgravity (McGrath *et al.*, 2002; Roth *et al.*, 2002). 2-D electrical resitivity tomography has been recently used to delineate vadose flowpaths in sinkholes (Schwartz and Schreiber, 2006). Smoke tests have been combined with geophysical techniques to map the vadose karst (Nyquist *et al.*, 2005) and determine if cavities are open or closed (Meighan *et al.*, 2006). Improvements in data interpretation are also being advanced (Nyquist *et al.*, 2007; Nyquist and Roth, 2005).
- The mechanics of sinkhole collapse have been modeled and illustrate the importance of recharge seepage pressure and over-pumping (Keqiang *et al.*, 2004). Physical models have been used to predict failure in cemented sands based on sand cohesion and thickness; and cavity geometry (Goodings and Abdulla, 2002).
- Leakage of water from dams built in karst areas is a well established problem (Milanovic, 2002). Research on this topic utilized coupled flow-geochemical models indicates that the increase in hydraulic gradient created by the dam drives the dissolution rates (Dreybrodt *et al.*, 2002; Romanov *et al.*, 2003).

U.S. MILITARY INSTALLATIONS ON KARST

Military bases typically have similar land uses as are found in metropolitan areas, such as residential areas, sewers, roadways, and airports. The major difference is military-specific use and management of ammunition and ordnance. The Department of Defense (DoD) has established the Defense Environmental Restoration Program (DERP) to address these issues. Within that structure, the Installation Restoration Program (IRP) is focused on health and safety impacts related to contamination. The DoD reports there are 4,200 properties in the IRP (U.S. Department of Defense, 2007).

A large number of military bases in the contiguous U.S. (more than 70) are located in karst regions (Fig. 2). All of the applied topics above – water quality, water quantity, geotechnical issues – are relevant to these sites. Many of the bases use karst water as their primary drinking water supply. At Fort Campbell Army Base, 40,000 customers use water from Boiling Spring (Fort Campbell, 2007). For bases being closed under the Base Realignment and Closure (BRAC) program, planning for land re-use requires knowledge of contaminants present, environmental liabilities, and vulnerable areas.

Most military installations that have well-developed karst have already taken their karst setting into account for water resource and environmental planning. Some installations in karst have used, or been part of, a basin-scale approach for water resources or environmental impact. Examples of these studies include extensive dye tracing to frame conceptual models and delineate ground-water basins at numerous karst facilities: Fort Knox Military Reservation, Kentucky (Connair and Murray, 2002), Fort Campbell, Kentucky/Tennessee (Arthur D. Little Inc., 1997), and Fort Leonard Wood Military Reservation, Missouri (Imes *et al.*, 1996; Kleeschulte and Imes, 1997). Other bases have incorporated a karst-approach to their regulatory investigation. For example, the Letterkenny Army Depot in southcentral Pennsylvania is listed on EPA's National Priorities List and is one of the 1995 BRAC sites (U.S. Environmental Protection Agency, 2007). Chlorinated VOCs have been detected in some springs on the facility. Karst springs were also found to discharge nitrates and RDX associated with ammunition burning at the Crane Naval Airfield Warfare Center in Indiana (DiGnazio et al., 1998; Krothe, 2003). San Antonio, on the edge of the karstic Edwards Aquifer, is home to multiple military facilities. One of those facilities, Camp Bullis, has conducted

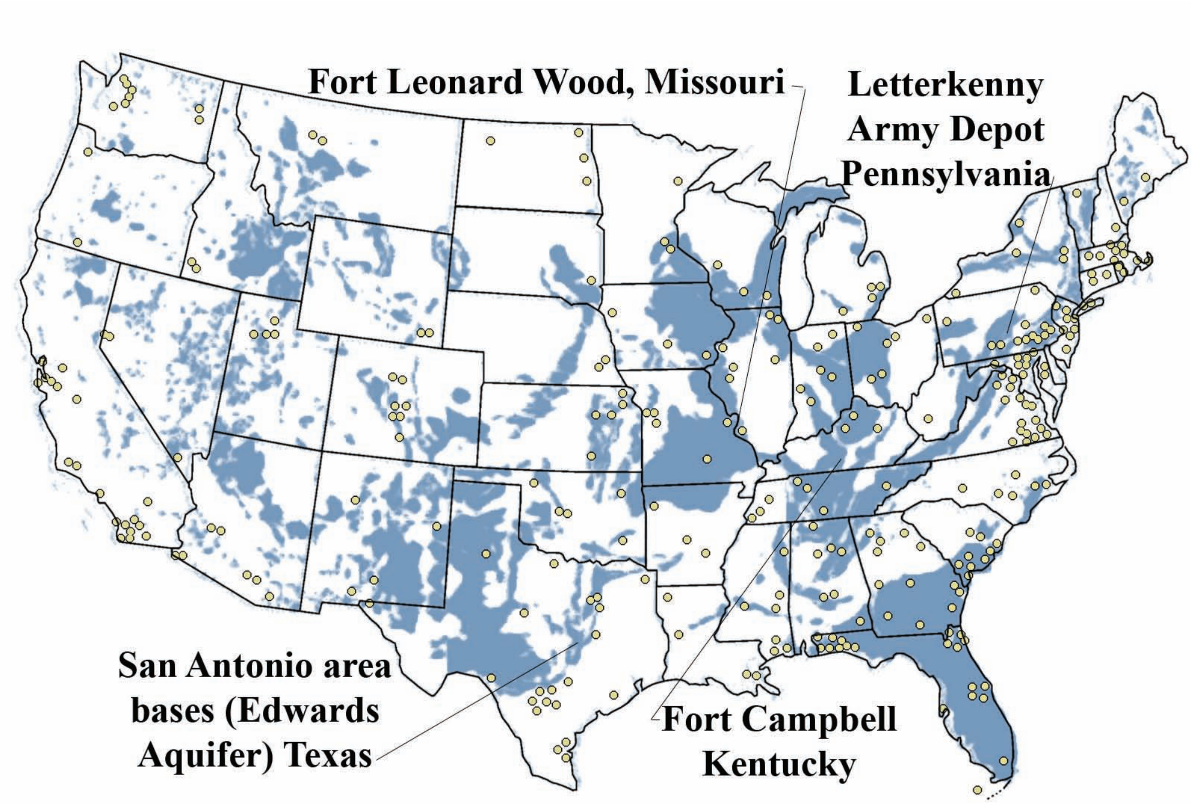

Figure 2. Location of karst regions (shaded) and military bases (dots) in the contiguous U.S. Locations of examples indicated. Modified from the National Karst Map and the National Park Service (National Cave and Karst Research Institute and U.S. Geological Survey, 2007; U.S. National Park Service, 2007)

integrated assessments of water resources, hydrogeology, caves and endangered species (Veni, 2004).

There are also U.S. military sites outside the country in which karst systems have been impacted. The Anderson Air Force Base in northern Guam is listed on the EPA National Priority List. Ground water is contaminated with lead, chlorinated solvents and toluene. The underlying North Guam Lens is the primary drinking water source for the area (U.S. Agency for Toxic Substances and Disease Registry, 2002).

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Part 3

FINDINGS AND RECOMMENDATIONS OF THE FOCUS GROUPS

FINDINGS AND RECOMMENDATIONS OF THE FOCUS GROUPS

The discussions of the Focus Groups were recorded as notes and on large paste-up sheets on the walls of the meeting room. Specific topics were identified and these were discussed in several iterations. At the end, the Focus Group leaders drew the main points together as the written reports in the sections that follow.

It will be noted that certain of these documents provide extensive additional bibliographies while others do not. The Focus Groups were encouraged to present their findings in any way that seemed appropriate.

The names of the Focus Group participants are listed with the reports. Their affiliations, addresses, and other contact information are given in the appendix.

Focus Group on Karst Hydrology – Conceptual Models, Aquifer Characterization, and Numerical Modeling

Group Participants: Martin Sauter, Leader. Matt Covington, Lee Florea, Franci Gabrovsek, Yongli Gao, Ronald Green, Jason Gulley, Russell Harmon, Ellen Herman, Pierre-Yves Jeannin, William K. Jones, Todd Kincaid, P.J. Moore, John Mylroie, Ira D. Sasowsky, Elizabeth Screaton, and Carol M. Wicks

INTRODUCTION

Flow of water in a karst catchment is mainly determined by the hydraulic gradient to a point of discharge (spring, river or coastline), the geometry of the karst features (fissures, fractures, conduits, and other zones of dissolution-enhanced high hydraulic conductivity), the sources (sinking streams, sinkholes, and the epikarst), and temporal variation of the recharge input. While the bulk hydraulic gradient can be determined in the field, the geometry of the karst features, their hydraulic parameters and their spatial distribution require considerable effort to quantify. Although some caves appear very spectacular and voluminous (if accessible), the fraction of rock occupied by caves in a karst system frequently is less than a few percent. This implies that these features are difficult to detect by drilling and even more difficult to parameterise hydraulically. Due to their important role in conducting water flow, both vertically in the vadose zone and horizontally in the phreatic zone, they cannot be neglected either (Klimchouk *et al.*, 2000).

In order to be able to predict flow through karst aquifers, it is critical to first design plausible conceptual models. Conceptual models provide a framework to support more quantitative mathematical models. Conceptual models are followed by a first principles understanding of ground water flow including quantized inputs and outputs. The final step is the quantitative mathematical or computer model. Such models allow, for example, the assessment of water resources, vulnerability of karst ground water to contaminants, potential flooding risks, and infiltration rates into caves.

In this report, different conceptualizations of karst systems are provided in order to convey an understanding of the different recommendations formulated for future research initiatives. The recommendation categories are assembled into different groups: processes, quantification of recharge and infiltration, characterization, models, and cross-disciplinary research needs.

CONCEPTUAL MODELS OF WATER FLOW IN KARST SYSTEMS

In recent decades, it has been realized that karst processes must be considered in a broader context than the traditional dissolution in circulating meteoric water. Karst, both surface landforms and caves, formed by water circulating downward (and also laterally) from a meteoric source on the land surface is referred to as *epigenetic* karst. Karst, mostly caves, formed by water migrating upward from depth is referred to as *hypogenetic* karst. Hypogenetic karst is often the product of dissolutional processes beyond the carbonic acid chemistry that is the primary driver for epigenetic karst, especially sulfur and sulfuric acid chemistry. Water often flows in epigenic karst aquifers in a turbulent regime. Flow in hypogenic systems is mostly laminar with caves embedded in a diffuse flow system that is completely decoupled from the surface hydrology. Models for karst aquifers developed in diagenetically mature, well-compacted carbonate rocks usually need to take account only of the conduit permeability and the fracture permeability. Matrix permeability is often very low although it is sometimes substantial. Such aquifers are referred to as *telogenetic* karst. Other aquifers develop is young carbonates where diagenetic processes may be incomplete. In *eogenetic* karst aquifers, matrix permeability is usually a dominant part of the flow system. Fractures may be a relatively minor feature.

Conceptual Models for Telogenetic Karst Aquifers

Most conceptual models of water flow in telogenetic karst distinguish three main zones (compartments) in the vertical direction. These are: the soil zone and epikarst, the unsaturated or vadose zone, and the phreatic zone. Although most conceptual models include similar structural features, the flow and storage processes assigned to them display large variations. Figure 1 presents a conceptual model of such a karst system.

The epikarst, a zone of increased weathering near the land surface, determines the distribution of recharge to a karst aquifer

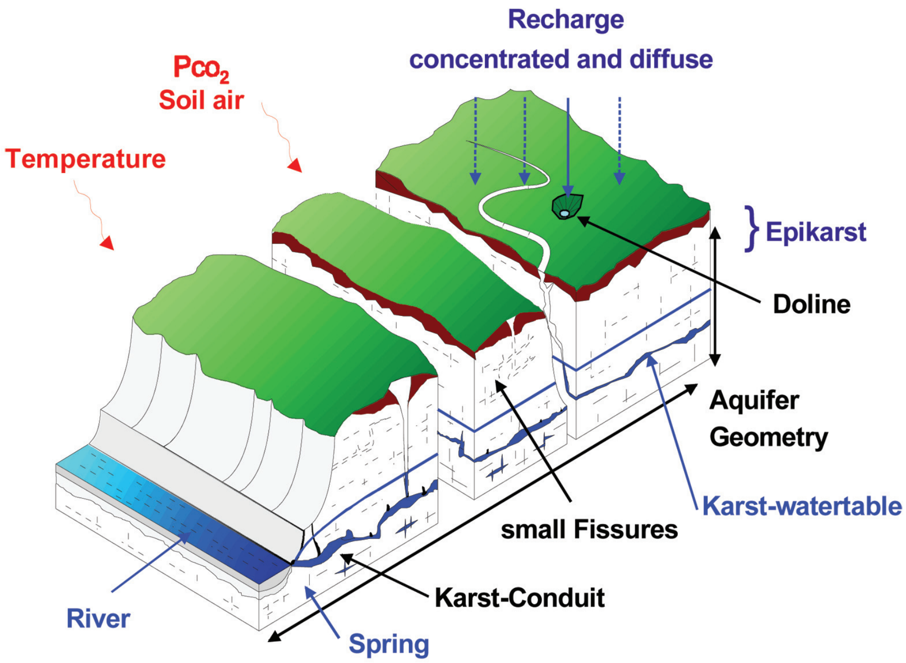

Figure 1. Conceptual model of a telogenic karst system in dense well-lithified carbonate rock (After Liedl and Sauter, 2003).

in both space and time. It can be visualised as a perched aquifer system channelling diffuse input towards shafts and sinkholes. The presence of the epikarst is believed to explain the highly heterogeneous water input to the system, both in space and time. Furthermore, it links climatic and near-surface geological conditions with the karstification of a limestone aquifer, defining both the hydraulic and the chemical boundary conditions for the development of the karst system. An understanding of the functioning of the epikarst is therefore a prerequisite for the quantification of infiltration (Jones *et al.*, 2003; Geyer *et al.*, 2008).

Due to the complex and heterogeneous nature of karst systems, quantification of recharge input is a major challenge. In order to be able to predict short term responses of karst systems to recharge events with numerical models, knowledge of the quantity of recharge, it's temporal and spatial variability and of the infiltration mechanism is a prerequisite.

Karst aquifers are characterized by highly varied hydraulic properties which are a result of the complex interactions between karst conduits, discrete fractures and the rock matrix. Conduits are characterized by low storage and high flow velocities, while the discrete fissured system and the rock matrix display much higher storage and low flow velocities. Due to this dual-porosity, dual-permeability structure of the carbonate medium the resulting hydraulic parameters are difficult to interpret from standard investigation techniques such as hydraulic tests, and cannot be easily regionalized at the catchment scale.

Conceptual Models for Eogenetic Karst Aquifers

Eogenetic aquifers, almost by definition, have high matrix permeabilities. The high matrix permeability creates a large accessible storage that is a significant contribution to the flow system. Flow in the matrix can be modelled as classical Darcian flow. However, it is the difficult to account for extensive conduit development in such aquifers, but the conduits are definitely present. Quantitative models must then address the interchange of conduit flow and matrix flow. Aquifers in the Paleozoic rocks of eastern United States are telogenetic karst as are many of the aquifers of Europe and the Mediterranean. Examples of Eogenetic karst are the Floridan Aquifer and the carbonate islands of the Caribbean.

In coastal regions, where carbonate rocks extend below sea level a circulating pattern of sea water and fresh water develops which enhances karst development in both dense and diagenetically immature limestones. The resulting cavities, known as halocline or flank margin caves are thought to contribute to reservoir porosity later in their geologic history (Sasowsky *et* *al.*, 2008). Flank margin caves have an entirely different role in aquifer hydrology than do the integrated conduit systems of telogenic karst.

Modeling efforts on flank margin caves has been undertaken but much remains to be done (Fig. 2)

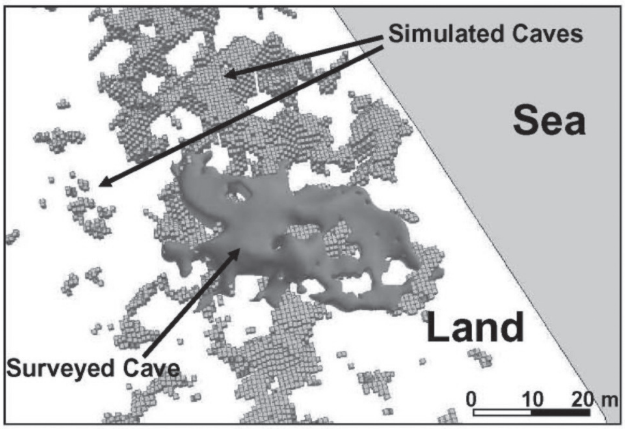

Figure 2. Computer generated model of a flank margin cave (box pattern) compared with a cave survey (smooth pattern). From Labourdette *et al*. (2007).

Conceptual Models for Hypogenetic Karst Systems

Karstification by rising deep-seated solutions has been recognized so recently that the development of conceptual models remains an open problem (Klimchouk, 2007). Unlike both telogenetic and eogenetic varieties of epigenetic karst where the chemical processes are moderately well understood, even the chemistry of hypogenetic karst poses many open questions. Some of the deep-seated solutions are derived from petroleum reservoirs, some are associated with volcanic activity, and some are deep-seated brines. For the most part, hypogenic karst flow systems are recognized only by caves that are later exposed. Active systems can be intercepted by drill holes, but both chemistry and flow hydraulics of the processes taking place at depth are poorly understood.

PROCESSES

A number of relevant flow, transport, to some extent reaction processes, have been implemented into the various types of models (discrete, hybrid, continuum models).

In a karst system, a number of different processes are superimposed so that the influence of individual processes is difficult to quantify, especially if their spatial and temporal variability is relevant. For example: The increase of discharge at springs can be attributed to: a) the specifics of storm intensity, spatial distribution and temporal variability, b) the characteristics of evapotranspiration at the soil – vegetation – atmosphere boundary, c) the heterogeneous infiltration process with rapid infiltration via fractures and sinkholes and slow infiltration through the vadose zone rock matrix, and d) the dualistic flow and storage processes in the phreatic zone (see also Smart and Hobbs, 1986) (Fig. 3). The analysis of spring discharge data therefore requires an appropriate conceptual model, the identification of the individual flow processes in the different compartments, as well as characterization of the geometry and hydraulic properties of the flow paths. Continuous measurement of the input functions such as precipitation and evapotranspiration is important.

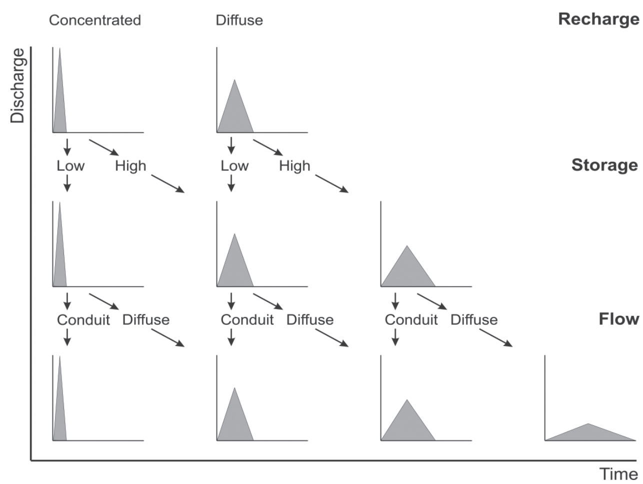

Figure 3. Schematic diagram to demonstrate the superposition of different processes and the influence of the different compartments on spring discharge (after Smart and Hobbs, 1986)

Further process understanding is required for the quantification of:

a) **Unsaturated flow**: Are film flow processes relevant?

b) **Open channel flow**: Is this process relevant at various flow conditions? Can certain observed thresholds, observed in the discharge pattern be explained by open channel flow conditions?

c) **Turbulent flow**: Although difficult to parameterize, turbulent flow processes can explain many observations in karst flow and transport (e.g. dispersion, tailings in tracer breakthrough curves). A quantitative and efficient approach in the identification and model implementation of turbulent flow processes is required. (Computational fluid dynamics models can simulate theses processes at laboratory scale, but the calculations are much more difficult at the catchment scale).

d) **Sediment transport**: What determines sediment transport; are there thresholds?

e) **Variable density flow**: Flow in island and coastal karst settings is determined, apart from the general characteristics of the carbonate limestone materials, by the variability in salt concentrations. Although in many circumstances, advective flow dominates flow behaviour there might be still-water environments, where convective processes might be important (e.g. for carbonate dissolution processes etc.).

f) **Multiphase flow**: Although important in the context of e.g. contaminant transport, multiphase flow processes will be difficult to identify and parameterise in this complex and highly heterogeneous environment. However, the development of models that incorporate multiphase flow processes (also air / water) might assist in understanding certain observations.

g) **Conduit-matrix exchange**, and the geochemical reactions that occur across this boundary, particularly in eogenetic karst.

Apart from the identification of processes and the characterisation of process parameters, it is also important to initiate investigations into an efficient implementation of these processes into the respective (discrete, hybrid, continuum) numerical models. To improve applicability of models to a larger segment of problems and the overall quality of model input, we need to improve our understanding of measurable parameters in karst systems and develop basic empirical relationships based on those parameters.

Badly needed are investigations into the system responses and thresholds. Extreme storm events are useful probes if aquifers are adequately instrumented so that complete response records can be obtained.

QUANTIFICATION OF RECHARGE INPUT AND INFILTRATION

Independent assessment of recharge is of prime importance for any model construction. Since recharge in karst aquifers occurs both spatially distributed (diffuse) as well as locally concentrated via sinking streams, shafts and dolines (discrete), substantial effort needs to be invested in its characterization. This includes:

a) **The quantification of total recharge at the soil base level**: Soils in karst regions are often very thin and frequently very patchy (often alternating between bare rock and soil pockets). Therefore, traditional classic soil moisture balance techniques do not necessary apply. Methods need to be developed, including hydrological, remote sensing and geophysical techniques, which take into account the specifics of karst soils.

b) **Characterization of flow and transport in the vadose zone**: The vadose zone, extending between the base of the epikarst and the ground water table frequently measures more than 50 metres, sometimes several hundreds of metres. The presence of this zone transforms the already complex recharge input signal into an even more complex one, composed of a rapid and a slow input with variable fractions and a variable temporal response. An integrated approach, using hydrogeological and geophysical characterization techniques is expected to provide at least some indications of the respective input functions.

These types of investigations are expected to be most successful if performed on a catchment scale. Quantitative measurement of input (precipitation) and output (spring discharge) can then be measured and an overall water balance established.

CHARACTERIZATION

The prime characteristic of telogenetic karst aquifers is, apart from their genetic history, the heterogeneity and the extreme contrast in the hydraulic parameters of highly conductive, but low storage, conduits and the low conductive but high storage fractured matrix. Although the conduit network dominates the flow pattern, it is extremely difficult to detect with drilling or traditional geophysical techniques because of its low volume fraction in the bulk aquifer. Therefore, active conduits cannot be properly characterized hydraulically or structurally, unless they are accessible for divers or located within the vadose portion of the cave system. Indeed, a fundamental limitation of all modelling efforts to date is that the conduit system must be put into the model "by hand". If the location and characteristics of the conduit system are completely unknown, any models and the corresponding model output will be of limited value.

Techniques need to be developed that provide the basis to locate individual conduits via novel geophysical or telemetric techniques and detailed mapping of hydraulic potential. Furthermore, cave mapping can be expected to provide analogues for submerged cave networks and the understanding of the genetic history of karst networks (geomorphological, paleokarst investigations) as well as numerical karst genesis modelling can supply additional information for the outline of the geometry of conduit networks.

In the case of hypogenic caves, the mere existence of a cave is difficult to determine as discrete recharge and discharge points are not available for sampling and measurement. Such caves can be quite large, for example, Carlsbad Caverns, yet have no obvious indication on the surface. Geophysical methods would also be useful, particularly if they could detect cavities at great depths.

MODELS AND MODEL DEVELOPMENT

A variety of modelling strategies have been developed. All show some promise but none are really satisfactory. The recent employment of lattice Boltzmann techniques for process modelling in karst appears promising. The overall goal should be process-oriented models that emphasize efficient numerical computation of large, catchment scale models, while honouring small scale heterogeneities.

Furthermore, since the different models require different model parameters (e.g. spatially averaged, discrete geometric, double continuum etc.) model adapted characterisation strategies need to be developed. The differences in parameterization strategies need to be highlighted and the relationship between actually measurable parameter (via hydraulic tests, etc.) and model calibrated parameters needs to be clearly stated.

COMMUNICATION AND CROSS-DISCIPLINARY RESEARCH

In order to further research into the hydrology, biology, ecology and geochemistry of karst, appropriate platforms need to be set up:

a) Provide a common language and understanding.
b) Organize joint experiments.
c) Provide common data bases (cave surveys, tracer experiments, hydraulic tests).
d) Identify suitable benchmark sites (karst catchments) for common interdisciplinary experiments. These catchments will:

i. Have long-term data records,
ii. Be relatively “simple” geologically and hydraulically
iii. Provide the basis for multidisciplinary research topics, e.g. the influence of microbial and biogeochemical processes on carbonate dissolution
iv. Be suitable, so that jointly (hydro-bio-chem) organised experiments have a chance to alter system behaviour.

CONCLUDING REMARKS

Although an obvious statement, it is important to reiterate that model selection needs to be based on the formulation of the problem to be solved. This requires a concise statement of the problem, an initial plausible conceptual model, the identification of relevant processes and parameterisation strategies.

Since wrong conclusions are frequently being drawn from erroneous model selection and interpretation of model results, it is suggested that a tool box/guidelines be developed for of sound karst modelling protocols. This means that guidelines should be formulated that suggest investigations and modelling strategies, based on the karst conceptual models and the problem in question. Benchmark catchments and sample calculations can demonstrate their application to real case studies.

Quantitative assessment of flow and transport in karst, the identification of important processes as well as the determination of process parameters among ground water professionals is generally hampered by the lack of understanding of the particulars of karst systems. It is important to convey to the professional public via appropriate media techniques (karst portal, etc.) that “karst is different.” It is also important to convey the expanded concepts with the distinction between epigenetic and hypogenetic karst and between telogenetic and eogenetic karst.

Focus Group on Geochemistry and Climate

Group Participants: Jay Banner, Leader. Penelope Boston, Liza Colucci, Brian Cowan, Amy Frappier, Cara Gentry, Russell S. Harmon, Brian Katz, Andrew Long, Jonathan B. Martin, MaryLynn Musgrove, Jud Partin, Jessica Rasmussen, Corinne Wong and William B. White

GEOCHEMISTRY OF MODERN KARST SYSTEMS

Importance to Science and Society

Water use management and water resource conservation practices require knowledge of aquifer functioning and processes. This is particularly true for karst groundwater resources, which are of enormous global economic value. Karst groundwater resources supply drinking water to an estimated 25% of the world's population (Ford and Williams, 1989). In the United States, karst covers approximately 20% of the land surface (Fig. 4) and provides substantial amounts of the groundwater used for drinking water. Karst aquifers and associated springs provide water for agriculture and aquiculture, drinking water, recreational and tourist opportunities, and habitat for myriad aquatic and terrestrial organisms.

Challenges to Water Resource Sustainability

Understanding of flow through karst aquifers, and the chemical and isotopic composition of the water clearly have high social relevance because of the susceptibility of karst to contamination through rapid infiltration (e.g., Field, 1988; Zuber and Motyka, 1994; Boyer and Pasqarell, 1995; Vaute *et al.*, 1997), and the impacts of karst processes on water resources. Fundamental karst processes such as karstification, speleogenesis, aquifer evolution, and sinkhole formation are all controlled by geochemical reactions (Dreybrodt, 1981; 1988; Beck, 1986; Palmer, 1991; Romanov *et al.*, 2003; Gabrovsek *et al.*, 2004). Detailed chemical and isotopic compositions of karst waters and their variability in time and space could provide additional insights into flow and reaction in karst aquifers. Challenges to water resource sustainability with respect to karst are numerous and are complicated by the inherent temporal variability of karst aquifers. Challenges include drought and flood vari-

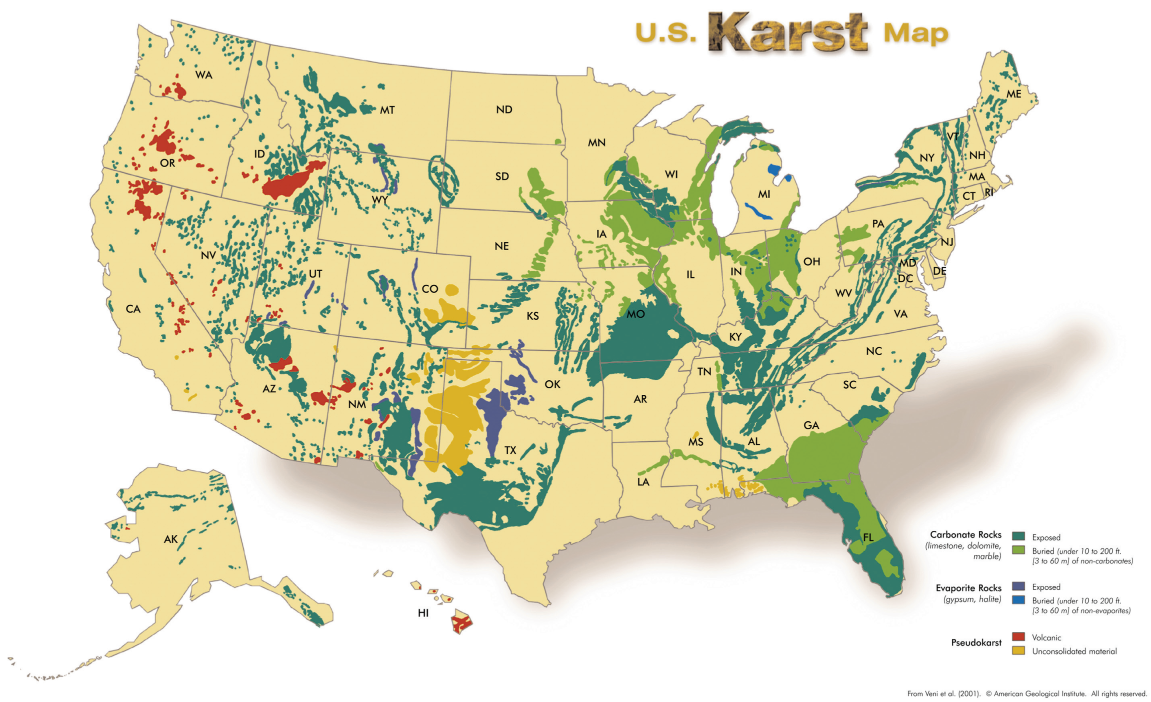

Figure 4. Karst areas of the U.S. Map provided by G. Veni and reproduced with permission of the American Geological Institute.

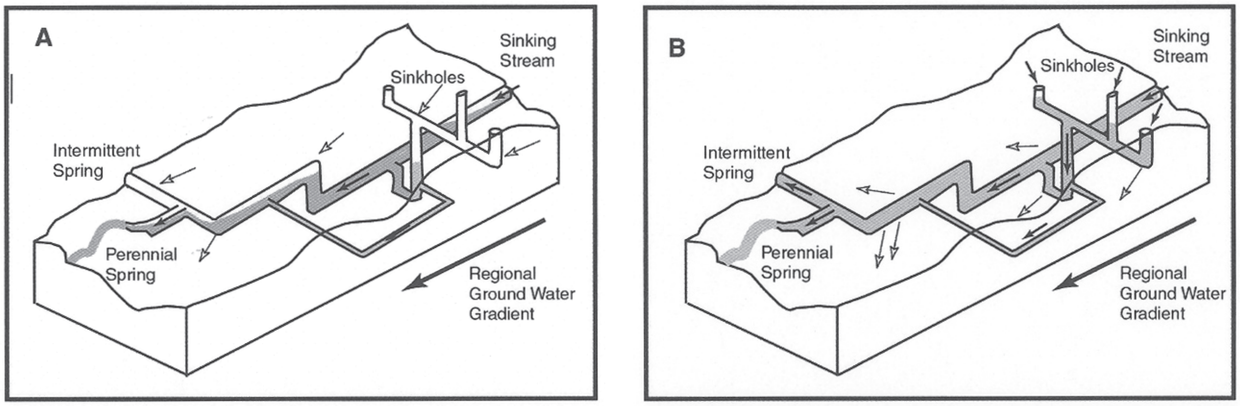

Figure 5. Schematic diagrammatic representation of the coupling of flow in conduits and through matrix porosity at A. Base flow conditions, B. Flood conditions. (From Martin and Screaton, 2001).

ability, residence time variability, aquifer contamination, and increasing demand for water resources.

Unique Vulnerability of Karst Systems to Contamination

Karst aquifers are highly productive sources of water, but many of the unique characteristics that render them productive also make them extremely vulnerable to contamination (Vesper *et al.*, 2001). Overlying soils are frequently thin to non-existent, providing little opportunity for filtration, sorption, or bacterial remediation of contaminants. Much of the infiltration is focused through discrete recharge features such as swallow holes and open fractures, which transmit water rapidly and provide minimal filtration. Within the aquifer, most of the transport occurs within a network of fractures and conduits. As a result, flow velocities are typically orders of magnitude higher than in porous media aquifers, sometimes as high as several kilometers per day. As a result of rapid flow velocities, ground-water flow can become turbulent, entraining particles and associated contaminants within the aquifer, and increasing turbidity and transport of hydrophobic contaminants, including pathogens. The rapid transport of water and contaminants from the surface through the aquifer to springs and wells may leave little time for response and/or remediation. Additionally, the extreme isotropy and heterogeneity of karst systems complicates prediction of transport velocities and even direction of flow, and leaves traditional contaminant transport models inadequate to address these unique vulnerabilities and complexities. Numerous human activities, including urbanization, agriculture, waste-water discharge, and deforestation have resulted in a variety of anthropogenic changes and contamination to karst aquifers, including nitrates and other nutrients, pesticides, solvents and other volatile organic compounds, pathogens (viruses, protozoa, bacteria), suspended sediment and turbidity, and pharmaceuticals (Carey and Lloyd, 1985; Boyer and Pasquarell, 1996; Katz *et al.*, 2001; Barrett and Williams, 1989; Pasquarell and Boyer, 1996; Mahler and Massei, 2007; Wolfe and Williams, 1999; Mahler *et al.*, 2000; Dussart *et al.*, 2003; Kuczynskaa *et al.*, 2003; O'Reilly *et al.*, 2007; Ryan and Meiman, 1996; Massei *et al.*, 2003; Drysdale *et al.*, 2004; Wicks *et al.*, 2004; Mahler, *et al.,* 2006).

KEY SCIENCE QUESTIONS

What is the extent, interconnectedness and structure of conduit vs. diffuse flow through karst aquifers?

One of the key challenges facing karst science is how to better understand and describe the extent and interconnectedness of conduit and diffuse flow. Karst aquifers can be considered to have dual or even triple porosity - a low permeability matrix, in which most of the storage occurs, and a conduit system, in which most of the transport occurs. A third type of porosity, micro-fractures with permeability intermediate between the matrix and the conduits, is often assumed as well.

Aquifer recharge can occur as focused recharge through discrete features such as swallow holes or open fractures, or as diffuse recharge through soils into the matrix. When no recharge is occurring, water stored in the matrix diffuses into the conduit system; when focused recharge occurs, higher head in the conduit system may result in diffusion from the conduits into the matrix. Thus most of the transport occurs through the conduit network, but under some conditions it is matrix water that is being transported, and under others it is surface water. Understanding of the different compartments of karst aquifers (e.g. conduit, fracture and intergranular/matrix porosity) has been improved through developments of deterministic and stochastic models of the aquifers. Deterministic models commonly use physical measurements (head, flow, temperature); some geochemical characteristics (e.g. specific conductance) can also provide constraints on models

The exchange and movement of water via conduit and diffuse flow plays a crucial role in determining aquifer recharge and storage, aquifer yield, and contaminant transport and storage processes in karst, and has significant bearing on understanding and modeling the hydrology of karst systems. Our understanding of the specifics and variability of conduit vs. diffuse flow in karst aquifers remains a significant topic of study, which is crucial for addressing quantification of the extent of conduit vs. diffuse flow, the controls of flow on the geochemical evolution of karst waters, differences in flow between the vadose and phreatic zones, temporal variability in vadose vs. diffuse flow under conditions such as storms, and aquifer response to stress (such as pumping).

What are the Mechanisms That Control the Chemical Evolution of Water Along a Flow Path From Surface Water to Ground Water and Return to the Surface?

The varieties of flow paths that characterize karst aquifers create a range of residence times for water from hours (Martin and Dean, 2001) to decades (Long and Putnam, 2004; 2006). These differences in residence times influence the chemical evolution of water, both in the water's major element chemistry through carbonate mineral reactions (e.g. Plummer, 1977), and also in its minor and trace element concentrations and isotopic compositions. In addition, contaminants introduced from recharge of surface water will react at varying rates and magnitudes with the rocks and surfaces of the aquifer materials, depending on their physical characteristics and mineralogy. For example, phosphorous can be a limiting nutrient for many karst spring systems, but also can take part in surface reactions with carbonate minerals and metal oxides. In contrast, nitrate, a widely recognized contaminant in karst systems because of the widespread application of fertilizers, undergoes very few reactions involving aquifer rocks.

What is the Fate and Transport of Redox-Sensitive Elements and Their Microbial Consequences and Feedbacks?

The role of microbes in geochemical functioning of karst aquifers is becoming increasingly clear through DNA characterizations of microbial communities in caves. These microbes may also influence the chemical and isotopic compositions of karst waters. Fractionation of carbon isotopes has been used as evidence of remineralization of organic carbon and the geochemical pathways of the microbial communities (McMahon and Chapelle, 1991). These pathways require terminal electron acceptors, which are commonly oxygen in karst systems because of the oxygen-rich atmospheres of many cave environments. Many other terminal electrons can be important, however, and there is recent evidence of microbially-mediated sulfur reactions in caves (Engel *et al.*, 2007). Additional terminal electron acceptors include metals such as iron and manganese. Oxidation-reduction (redox) reactions provide intermediate amounts of energy to carbon-oxidizing microbes and are likely to take place on a continuum of reactions between oxygen and sulfate and carbonate reduction (Froehlich, 1979). Cycling of metals is readily observed as coatings of iron and manganese on cave surfaces (e.g. goethite, limonite, and birnessite) and their precipitation can incorporate a range of other elements (Ba, Sr, P), thereby impacting the overall chemical composition of karst waters. The concentration of oxygen and other terminal electron acceptors may range widely throughout an aquifer, particularly outside of cave environments. Redox reactions may also be important in microenvironments of the epikarst and in matrix fracture and primary microporosity, where water can be separated from the atmosphere, allowing oxygen to be quickly consumed. Focused geochemical studies of these environments and water associated with different karst conditions may provide for improved understanding of the role of microbial processes.

What are the Impacts of Land Use Change and Climate Change on Water Resources and Can the Impacts Be Distinguished?

Land use changes have significant impacts on karst areas. Anthropogenic activities such as urbanization and development, deforestation, agriculture, ranching, livestock grazing, fire suppression practices, urban landscaping, landfills, waste-water discharge, sewage disposal and sewage and municipal water infrastructure can all change the nature of land surface and water resource interaction (e.g., Boyer and Pasquarell, 1999; Drew, 1996; Garcia-Fresca, *et al.*, 2004; Harding and Ford, 1993; Parise and Pascali, 2003; Sauro, 1993; Wang *et al.*, 2004; Williams, 1993). Negative impacts such as soil degradation, groundwater salinization, increases in contaminant concentrations, degradation of water quality, ecosystem and biodiversity loss, and changes in aquifer storage, recharge, and water availability can result in karst regions as a result of land use changes.

The impact of climate change on karst and karst resources represents an emerging multifaceted question. Climate change may significantly affect precipitation amounts and seasonality, water availability, river runoff and streamflow, and frequency and intensity of droughts and floods (IPCC, 1997, 2001, 2007). The intersection of anticipated climate change impacts and the inherent temporal variability of karst water resources necessitate a better understanding of climate change impacts in karst regions. There is, however, a lack of information on 1) the mechanisms and timescales at which land use changes and climate change impact karst aquifers, 2) if these changes are linear or threshold induced, and 3) possibilities of reversibility and/or remediation of such changes. This information is essential for water resource management and planning, and for establishing sustainable management practices. Effects of land use change and climate change on water resources may be synergistic, but measuring and distinguishing them presents a significant challenge. Paired or comparative studies of watersheds, regions, or aquifers may provide insight, as may predictive forecasting. The integration of hydrologic and regional scale climate models is a developing research direction (Gulden *et al.*, 2007; Niu *et al.*, 2007) that will have direct application to karst systems.

HOW TO ADDRESS THESE KEY SCIENCE QUESTIONS

High-Resolution Temporal and Spatial Analysis of Geochemical and Physical Variability, Particularly for Extreme Events (Floods, Droughts, etc.).

Information gained from high resolution temporal and spatial analysis of geochemical and physical variability, particularly for transient events such as floods, will be extremely valuable for improving our understanding of karst systems. Numerous studies have demonstrated that following rainfall, large changes in water level, spring discharge, turbidity, and concentrations of natural and anthropogenic chemical constituents may occur at a time scale of hours (Fig. 6) (Ryan and Meiman, 1996; Mahler, 1997; Mahler *et al.*, 2000; Massei *et al.*, 2002). For example, concentrations of atrazine in a karst spring in Austin, Texas, increased by a factor of more than 50 in the 20 hours following rainfall (Mahler and Van Metre, 2000). Thus, monitoring and models designed for time steps of months or even weeks are likely to fail to detect the dynamic responses of karst systems. Analysis of breakthrough curves can provide information such as transport times, dilution factors, location of contaminant sources, and diffusion factors, and are critical for calibrating transport models.

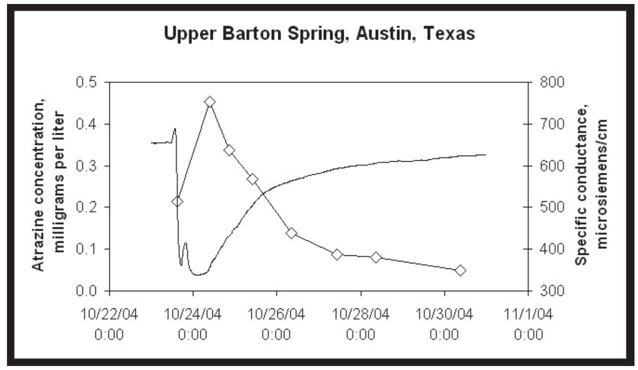

Figure 6. Rapid changes in specific conductance (solid line) and atrazine concentration (diamonds) reflect the influx of surface water to Upper Barton Spring, Austin, TX, following a storm. Adapted from Mahler and Massei (2007).

Because of the extreme heterogeneity of karst systems, data collected at high spatial resolution is valuable. In general, monitoring of springs should be considered a first priority, as springs act as integrators of the entire karst system (Quinlan, 1989). Even wells within a few meters of one another can show significantly different responses in piezometric level and geochemistry (e.g., Mahler *et al.*, 2000). This piezometric instability, in turn, can be extremely useful in investigating the spatial heterogeneity inherent in karst (Palmer, 2002).

Integrate Geochemical and Physical Information to Enhance Current Modeling Capabilities

Understanding the complexity of karst geochemical and physical dynamics requires the use of multiple integrated techniques. Modeling geochemical and hydrologic signals and responses is particularly effective in karst aquifers through applications such as lumped-parameter modeling (deconvolution) and frequency or wavelet analyses (Long and Putnam, 2004; Massei *et al.*, 2006). These methods are useful for understanding and quantifying geochemical and physical processes of karst systems, particularly those related to conduit and diffuse flow. Recently developed models for interpreting age-dating tracers (CFCs, tritium, SF6, 85Kr, 36Cl) for karst aquifers are useful for estimating the residence times of conduit and diffuse flow and the characteristic responses of these distinct components (Long and Putnam, 2006). These methods could be enhanced further by incorporating a 14C data component. Application of specialized tools such as these with common geochemical models such as NETPATH and PHREEQC (Plummer *et al.*, 1992; Parkhurst and Appelo, 1999) could result in robust approaches to understanding karst processes. Modeling geochemical processes in conjunction with fluid flow is a frontier in hydrologic modeling and would be particularly useful when considering karst dynamics. Application of advanced parameter estimation methods (Doherty, 2002) can be used to further the science of modeling karst aquifers through more efficient data processing and objective quantification of aquifer properties.

Apply Newly-Developed Techniques and Explore Additional Applications of Existing Techniques.

The integration of multiple tracers in an aquifer system may provide more robust and/or complementary information regarding tracer behavior and geochemical and aquifer processes. A large number of potential anthropogenic and natural tracers are available to assess mechanisms of groundwater geochemical evolution; many are underdeveloped or poorly understood due to complexities in tracer behavior. Natural geochemical tracers such as trace element ratios and stable isotopes historically have been applied to address karst groundwater evolution processes, mineral solution equilibria, ground water flowpaths, surface water – ground water interactions, hydrograph separation, and sediment transport. Anthropogenic contaminants in karst systems provide opportunities to develop new tracers that may provide specific details of contaminant transport (e.g., Mahler and Massei, 2007). Novel applications of radiogenic isotopes, such as Sr isotopes (Musgrove and Banner, 2004; DeMott *et al.*, 2006) may provide insight into processes of soil contributions to groundwater geochemistry, land use and urbanization impacts in water resources, groundwater mixing processes, and sources of dissolved constituents to groundwaters. Soil profiles of chloride and tritium can be used to estimate past recharge rates over tens and, in some cases, hundreds of years (Herczeg and Edmunds, 1999) with implications for water balance questions and resource sustainability. Applications of tracers such as radiogenic isotopes, rare-earth elements, Fe isotopes, and biomarkers are frontiers in karst geochemistry.

The vulnerability of karst aquifers to contamination is exacerbated by recharge of surface water into underlying aquifers (Katz *et al.*, 1995). These processes can result in groundwater-quality problems, such as high concentrations of iron and hydrogen sulfide, and undesirable bacteria, protozoa, and fungi (Krause, 1979; McConnell and Hacke, 1993; Katz *et al.*, 1995; Plummer *et al.*, 1998). The combined use of various geochemical tracers, including radiogenic and stable isotopes, tannic acid, chloride, and silica have been effective in calculating the proportion of river water that mixed with groundwater (Katz *et al.*, 1997, 1998). Blackwater streams are common in the southeastern United States and can introduce large amounts of natural organic matter (NOM) that can produce disinfection byproducts (DBPs) during drinking water treatment (e.g. disinfection with chlorine). Understanding the geochemical factors involved in the degradation or alteration of NOM in the environment is critical for accurate prediction of the formation of DBPs, such as trihalomethanes and other contaminants (e.g., Rostad *et al.*, 2000).

GAPS IN THE PRESENT RESEARCH INFRASTRUCTURE

Responses in a karst system can be long term and short term. Long-term (years to centuries) and short-term (hours to weeks) time-series data are useful for understanding these varied responses. Such data can be acquired if funding priority is given to establishing intensive monitoring networks in karst systems. Long-term data collection efforts for geochemical tracers should be conducted similar to the way hydraulic data is typically collected (e.g., streamflow, spring flow, and hydraulic head). NSF’s proposed WATERS Network would help achieve this goal. Furthermore, analysis of the geochemical response of karst systems to changes in the landscape and ecosystems that are developed in karst terrains would permit a deeper process-level understanding of the functioning of karst hydrology. To acquire these data would require an intensive monitoring system such as that proposed for the National Ecological Observatory Network. Analytical advances in terms of isotopic analysis of specific organic compounds in water, portable chemical and isotopic analysis via methods such as Raman spectroscopy and mini-differential optical absorption spectrometry will be required for the next step in *in situ* monitoring of karst flow systems. Small, secure, mobile, inexpensive, and durable instrumentation to automatically collect a range of geochemical and physical data in multiple aquifer components (e.g., soil, vadose, phreatic, epikarst, conduit porosity, diffuse porosity, along flowpaths) at high resolution will be a critical step in addressing research needs.

As karst science grows into an integrated field of hydrologic, modeling, geochemical, biological, and ecosystem disciplines, a need exists for storing and sharing of geochemical and physical data from karst systems. A data portal, or central system for data information sharing and discussion, beyond the published scientific literature was recognized as a necessity for future integrative efforts in karst science in order to address larger collective goals. Additionally, beyond the karst community, a need for improved cross-disciplinary communication and collaboration with the broader geochemistry and climate community exists, to include scientists with expertise in ecosystem function, global atmospheric and marine and climate systems. Improved interaction and communication with the public is a continuing need.

CLIMATE CHANGE AND KARST SYSTEMS

The observed warming trend over the last century has motivated extensive research of the interactions between temperature and precipitation variability in past, present and future climates. As detailed in the 2007 IPCC report, understanding where, when and why dramatic variability occurs is critical to societies managing food and water resources in climatically marginal regions, such as northern China and the African Sahel (Menon *et al.*, 2002; Nicholson *et al.*, 1998). Karst system science can offer a unique perspective on the temporal and spatial links between climate and hydrology. Given that karst ground water is an important component of the world's drinking water, karst systems are of central importance to water quality and water availability issues.

CLIMATE CHANGE – KEY QUESTIONS

What is the Relationship Between Climate Change and Water Availability?

The mechanisms driving high-frequency paleoclimate variability in climatically-marginal regions are not well known due to the general scarcity of long-term, high-resolution, precisely dateable proxies for precipitation in continental interiors. Speleothems have the potential to provide this information through multiple proxies including geochemical data, physical proxy data such as annual banding, and growth rate (Musgrove *et al.*, 2001; Asmerom and Polyak, 2004). Given the large spatial and temporal distribution of speleothems, these studies can provide unprecedented insight to:

- The underlying mechanisms that have modulated past climate variability across multiple timescales, from seasonal to millennial (Asmerom *et al.*, 2007).
- The frequency and magnitude of extreme events such as droughts, floods, and cyclones (Frappier *et al.*, 2007).
- The implications of past variability on future climate change.

How is Climate Variability Expressed in Terrestrial Environments?

The pursuit of paleoclimate reconstruction has centered on excellent marine and ice core records, there are significant gaps in our understanding of climate change in terrestrial environments. Speleothems can provide significant advances in this area, both in terms of relatively long-term, astronomically forced changes, to abrupt climate change, such as those driven by changes in ocean circulation, to monsoonal and El Nino-Southern Oscillation phenomena. One example of this potential is a speleothem record from Hulu Cave, China, which shows clear connections between atmospheric greenhouse gas concentrations and temperatures, as determined from ice core records, and Asian monsoon intensity as reflected in speleothem oxygen isotope variations (Wang *et al.*, 2001).

What are Feedbacks Between the Global Carbon Cycle and Karst Systems?

Carbonate rocks are a relatively soluble and large source of carbon that constitutes a significant component of the global biogeochemical cycle of carbon. Understanding the role of karst in the global carbon cycle will require studies of the feedbacks between changes in sea level with changes in exposure, weathering, and production of calcium carbonate in coastal regions. Studies will be needed that can address the timescales on which carbon is sequestered and released in terrestrial systems. In particular, these studies will need to address the question "To what extent are karst systems a source or sink for CO_2?" Key constraints on karst-carbon cycle feedbacks will come from an improved understanding of the rates of karst geomorphic development relative to temporal changes in sea level, atmospheric composition and rainfall amounts.

What are Feedbacks Between the Local Carbon Cycle and Karst Systems?

Carbon cycling at the landscape level may be significantly affected by interactions between the surface and underlying karst. The transport of carbon out of soils and into the karst environment is a flux that is rarely treated in carbon-cycle modeling. In the opposite direction, the movement of CO_2 from caves, springs and wells has not been documented in a comprehensive manner at the landscape scale. This flux is also important in modeling speleogenesis and subterranean calcite deposition.

HOW TO ADDRESS THESE KEY SCIENCE QUESTIONS

Many of the questions outlined above can be addressed through the development and integration of speleothem records that are well-dated, employ multiple proxies, and come from a wide range of geographic locations that span multiple scales. This may be similar to the approach of constructing and compiling marine and lake records to produce the CLIMAP data set. Significant resources would be required to build a "SPELEOMAP" data set.

Essential to reconstruction of past climate change using speleothems is a rigorous evaluation of and calibration of what the elemental, isotopic, and growth rate proxies represent. Three main approaches may be taken toward this end: 1) comparison of speleothem proxies to multiple independent records of climate change over the same time interval in the same region; 2) growth of speleothems under controlled laboratory conditions in which a range of parameters can be varied; and 3) growth of speleothems in modern karst environments in which environmental parameters can be monitored.

GAPS IN THE PRESENT RESEARCH INFRASTRUCTURE

Network of Intensive Monitoring of Modern System for Assessment and Calibration of Speleothem Proxies

Following on the discussion in the section above, the evaluation and calibration of speleothem proxies must include the assessment of equilibrium precipitation of speleothem calcite (Mickler *et al.*, 2004, Banner *et al.*, 2007). The most unequivocal means to achieve this assessment is through the analysis of the modern system, which can be accomplished through establishing monitoring networks in karst aquifers. Understanding of temporal changes of the modern landscape above karst aquifers and the modern aquifer system are going to be essential for interpreting seasonal to millennial scale records in the past. This will require studies of changes 1) above caves (thickness, moisture, water chemistry, productivity of soils, vegetation, and weather, 2) at cave drip sites (cave-air meteorology, drip rate, drip composition, and 3) event sampling. Studies of human-driven land-use change in karst systems will make speleothem studies more relevant to human time scales. This will require an even more extensive monitoring system and research infrastructure funding than that described for geochemical monitoring. It will be necessary to monitor 1) the local weather, hydrology, ecosystem and landscape above a monitoring cave system; and 2) *in-situ* cave measurements: cave meteorology and air composition, water flow, water chemistry, calcite growth, and calcite chemistry.

Standardization, Archiving, and Conservation

Standardization of several practices in using speleothems for climate reconstructions can help to yield not only better quality datasets, but will help preserve cave environments for future generations. Preliminary reconnaissance-type field missions that employ minimally-invasive sampling can help to better target specific samples while preserving cave formations. Minimally-invasive sampling can be done via small cores to determine age ranges and suitability for geochronology of multiple speleothems without removing them from the cave environment. Samples may also be imaged by computed X-ray tomography to determine if they are petrologically suitable, and then replaced in the cave environment if deemed unsuitable for further study (Mickler *et al.*, 2004b). At present, funding agencies will typically not fund projects of this design because of the length of time they would cover. A renewed emphasis on small grants reserved for exploratory, or reconnaissance, research would help promote minimally invasive sampling. Also, a central storage facility of samples, either virtual or real, such as those already employed by the ice core and sediment core communities, can help to minimize sample collection where research group interests overlap. To obtain a sample from this facility, a research group would need to write a short proposal about the project and how the samples can address those problems raised. Lastly, a central archive of not only proxy data collected, but metadata about sample selection processes as well, will help future studies produce better quality datasets.
Replicate records in multiple samples from the same cave as well as in caves across a region would help validate and facilitate interpretations of karst paleoclimate reconstructions (Musgrove *et al.*, 2001; Wang *et al.*, 2001; Treble *et al.*, 2005; Williams *et al.*, 2005; Johnson *et al.*, 2006; Wang *et al.*, 2006). This helps to separate hydrologic processes from climatic processes and strengthens climatic interpretations drawn from the records. While this may introduce an initial higher cost, it will reduce final costs by producing reliable records which do not need replication. A better spatial coverage of speleothem reconstructions can also addresses local versus regional-scale climate changes, much like modern satellite datasets.

Working in karst environments without adversely affecting them is a major challenge in cave research and conservation. Is it possible to enact an international research policy regarding cave research that scientists will follow? The varying factors of different local and federal restrictions on cave access and research permitting is an obstacle to be addressed. One aspect of such a policy might be the coring and reconnaissance analysis of speleothems vs. the multiple whole sample removal from caves. The karst community lacks an archival system for researchers such as that which exists for ice cores, yet speleothems are rarer. There are competing pressures of multiple sampling trips needed if reconnaissance sampling is employed, vs. the time and funding required for the recon approach. One way to enact such a policy, if it could be agreed upon, would be to require a conservation plan as part of the “Broader Impacts” section of a research grant proposal.

Geochronology

The road to high-fidelity, well-dated speleothem paleoclimate records is paved with more dates than at first appearance might be necessary, and more reproducibility than is often funded or published. This will require laborious, high-resolution reconstructions of 20th century climate and, absent visible banding, identifying chemical, isotopic, or petrographic proxies that occur in annual cycles, and counting these annual cycles back from the present. The methodology for executing such records exists or can be developed, but it will require significant effort that can be driven by funding priorities. To improve the accuracy of U-series age determinations, we will need an improved understanding of initial $^{230}Th/^{232}Th$ values incorporated into speleothems. Studies of modern system, zero-age speleothems, akin to studies of living corals to constrain marine initial $^{230}Th/^{232}Th$, will be valuable for addressing this issue.

SUMMARY OF RESEARCH NEEDS

In the next decade, the study of speleothems will have a large impact in two areas of the earth sciences: (1) the understanding of the major factors that have caused Earth's climate to change in the past, and that likely will cause Earth's climate to change in the future, and (2) the understanding of those soil, vadose zone, cave, aquifer and climatic processes that affect the isotopic and chemical composition of karst waters and the cave deposits that precipitate from them. Important climate science goals will relate to the first point, whereas some significant understanding of the second set of processes will be necessary in order to fully realize the first set of goals. The improved understanding of the second set of processes will have direct application to important science questions regarding the operation of modern karst systems.

CROSS-CUTTING THEMES WITH OTHER DISCIPLINES

Karst research has synergy with and bearing on other fields of study including landscape ecology, microbial ecology; the global carbon cycle; biogeochemical cycles; water resource policy and decision support systems. Some of the links between these fields and karst systems are discussed above, such as in the section on the feedbacks between the global carbon cycle and karst systems. Other links are posed by the following questions that cross cut different disciplines represented in the Karst Research Frontiers workshop:

- What role do microbes play in calcite precipitation or dissolution and speleothem growth? What role do microbes play in incorporation of proxies in speleothem records (e.g. C-isotopes)?
- What geochemical data and spatial/temporal resolution are needed for testing and advancing hydrologic modeling of karst systems?
- How do changes in ecosystem functioning, both within a cave and on the landscape above a cave, impact speleothem growth and incorporation of inorganic and biological proxies?
- How can decision support systems be developed to maximize benefits of karst resources to stakeholders while preserving the natural capital of karst terrains?

Focus Group on Caves and Karst as Model Systems in Geomicrobiology

Group Participants: Annette Summers Engel and Diana Northup, Leaders. Marcus Gary, Brett Gonzalez, Juan Gonzalez, Elena Hutchens, Dan Jones, Jennifer Macalady, John Spear, and Michael Spilde

INTRODUCTION

Microbial life evolved between 3.5 and 4.0 billion years ago and evidence suggests that microbes have played essential and ubiquitous roles in geological and ecological processes through time. In particular, most low temperature geochemical reactions are controlled, to a large extent, by microbial metabolic processes. Through metabolic, enzymatic, or cellular catalysis, microbes participate in the precipitation of minerals and influence rock dissolution, thereby shaping and changing the earth's landscapes through time. From an ecological standpoint, microbes serve as the energetic base of ecosystems, serving as a food source for higher trophic levels, and also producing ample, high quality energy through autotrophic metabolic pathways.

Karst landscapes comprise 12.5% of the earth's ice-free land surface, coinciding with the global occurrence of carbonate sedimentary rocks. Consequently, karst surfaces offer exceptionally reactive interfaces for microbially induced and enhanced reactions occurring at air-rock-water interfaces. Considering the extent to which carbonate rocks comprise the rock record on earth and the depths to which karstification occurs in the subsurface, the microbial biomass within karst settings and at karst interfaces is potentially tremendous. Therefore, microbes and microbial processes are central to all of the karst-related sciences.

In general, microbial scientists (e.g., microbial ecologists, microbial taxonomists, microbiologists, astrobiologists, and paleontologists) aim to understand the diversity of microbial life on earth, as well as how and when life evolved. Another goal for some microbial scientists (e.g., including geomicrobiologists, geobiologists, and geologists) is to be able to discern between biotic and abiotic processes in modern and ancient environments, and the extent to which microbes have influenced geochemical and geological processes, as well as how those processes have affected the diversity of microbes. Because ~1.6 billion people depend upon the health of karst terrains and aquifers for their water supply world-wide (Williams, 1993), other types of microbial scientists (e.g., biogeochemists, organic geochemists, environmental and civil engineers, and hydrogeologists) want to know how microbial processes alter the quality of water, the atmosphere, and the environment in general, as environmental conditions may affect human health.

Cave and karst settings are model systems where these research goals can be easily and readily addressed by microbial scientists. From a microbial science standpoint, caves and karst are unique to study because of the link between the surface and the subsurface (i.e. the so-called, "critical zone"), as well as the longevity of some karst settings. Based on in-depth discussions by Workshop participants, three main research themes and associated questions emerged as priority future research areas. These research directions should yield promising practical (i.e. economic) and intellectual results, as well as provide avenues for prosperous educational and outreach endeavors. Additionally, one key hope was that future investigations of microbial processes and activities in cave and karst settings should involve multidisciplinary and international collaborations. The remainder of this report details the consensus of these discussions and presents recommendations for cave and karst microbial science future research directions.

MAIN RESEARCH THEMES

Breakout session discussions centered on three main research themes, prompted by the State-of-the-Art presentations. Focus questions are presented with commentary, additional questions, and future goals to focus the question.

Biodiversity and Evolution

a) Who's Home? All three domains of life (Eukarya, Bacteria, and Archaea) and viruses occur as microscopic life in caves and karst. Among the Archaea and Bacteria, abundant novel organisms representing new genera, families, and orders have been previously uncovered from caves and karst using culture-based and molecular phylogenetic studies. For many different cave habitats, such as acidic cave-wall biofilms and surfaces, iron and manganese crusts on cave-walls, or microbial mats from sulfidic cave streams, the question, "Who's home?", is getting answered . Some caves are hotspots of microbial biodiversity, with a prime example being associated with sulfidic caves and karst springs. Basic microbial biodiversity studies based on 16S rRNA gene surveys and phylogenies have shown that there are community and species parallels among caves on different continents. These habitats provide a model set of habitat conditions for investigating biogeography and addressing the question of whether "everything is everywhere; the environment selects."

However, vast gaps still remain in our knowledge of microbial diversity in caves and karst, as most of the previous studies have been done in only a few caves. There is a fundamental need to explore and document the biodiversity of different kinds of habitats in caves and karst systems. More baseline data are needed to determine if microbes are endemic to caves or karst, or the subsurface in general, and if so, in what numbers. Almost nothing is known about viruses in karst. The need to know more about habitat biodiversity is not unique to caves and karst studies, but is perhaps more acute because of the overall societal interest in understanding karst processes. Increasing our understanding of cave and karst biodiversity allows for better protection and conservation of these novel communities.

b) What is the Source of Microbes in Caves and Karst? Almost a century ago, biospeleologists understood that microbes were transported into cave systems via dripping water, air currents, animals, and human visitors. Some cave microbes were found to be merely a subset of microbial groups from the surface that are known to be associated with overlying soil, including organic matter and detritus from plants, rhizosphere exudates, etc. From an ecosystem perspective, the metabolic types of allochthonous microbes can have a profound impact on the niche availability and the overall function of the ecosystem. From a geological perspective, microbial life has a long history of existing and interacting in mineral environments and it may be necessary to consider that the depositional history and geological setting of the host rock in sourcing microbes in cave and karst. The discovery of chemolithoautotrophic communities in caves has opened up a new suite of questions about the origin of these microbial communities. Phylogenetic reconstruction of 16S rRNA genes suggests that cave microorganisms from Lechuguilla Cave in New Mexico have marine ancestors. Most limestones, including the limestone in which Lechuguilla Cave formed, are marine in origin.

Several questions arose from discussions about the origin of microbes in caves and karst, and the implications of conducting research in cave and karst settings that could influence our understanding of microbial evolution and speciation hypotheses:

- How do microorganisms speciate in the subsurface? Is gene transfer as active in the subsurface?
- Could microorganisms identified from caves, and others in similar subsurface environments, have existed in this milieu for millions of years, slowly reproducing?
- Does the depositional setting of the original host rock influence the modern microbial communities found in the subsurface?
- Does allopatry influence the formation of new subsurface microbial species, especially in caves that are not well connected hydrologically to other voids?

Basic research is needed in the whole of the microbial sciences with respect to mechanisms by which speciation can be due to gene-swapping (including viral) interactions, and general ecosystem services by viruses.

c) Do Microbes Adapt to the Subsurface Environment? Caves are often regarded as being very stable in temperature, light conditions, and relative humidity. However, there are aspects of caves that can change rapidly, especially in hydrologically active systems (e.g., caves that flood seasonally or periodically). Ranges of physiochemical environments and habitats not only dictate the types of speleogenetic processes that might be prevalent, but also the different metabolic strategies that microorganisms might need given that particular set of parameters. In karst, these parameters could be related to the speciation and availability of redox-sensitive elements, or the types and loading of carbon or other nutrient sources. Aquatic habitat conditions will be distinct from subaerial conditions and stresses, as microbial community composition and structure from subaqueous microbial mats are different than the composition and structure of communities living attached to cave-wall mineral surfaces. In more hydrologically stable areas (e.g., within the phreatic zone), there are no obvious "hot times" in microbial community development. But, in caves like Cueva de Villa Luz in Mexico, with large and erratic inputs of reduced gases, the hypothesis would be that diversity may vary over time. Does the ingress of reduced gases into cave environments bring with it new microbial biomass that originates from deep within the Earth's plumbing?

Do surface-derived microorganisms die due to the oligotrophic nature of many caves, becoming food for higher level organisms, or can the surface-derived microbes function in the restricted (i.e. based on physicochemical conditions) habitat and subsequently colonize caves? If so, then how do these microbes, once exposed to the harsh and erratic surface conditions, adapt to the more stable conditions of karst? Unlike surface-derived transport in dripwaters, it is unclear the extent to which air currents and ground waters bring microbes into the cave environment. For this research to reach conclusions, the geological and hydrological connectivity that exists, and has existed, between surface and subsurface environments needs to be better characterized. In the more stable, low-nutrient (i.e. low organic carbon) habitats, selection may lead to the streamlining of genomes and possibly to a reduction in genome size. Fundamental questions need to be addressed about evolutionary forces in the subsurface:

- Are microbes adapting to subsurface conditions?
- At what rate does adaptation proceed in the subsurface?
- How do microbial mutation rates compare between surface and subsurface environments?
- Which is faster: genetic change or transport of microbes?

Investigation of these questions will be greatly aided by genomics and whole genome sequencing of communities of microorganisms in the subsurface. Thus far, whole genome sequencing for cave microbes has not been done. However, we anticipate that the rapid advances in the field, from addressing the basic questions, “Who’s home?” to “Why are these microbes in this cave?” will be completed and will establish some evolutionary hypotheses. Whole genomes of microbes from similar karst habitats, possibly distributed throughout global systems, will be relevant to fundamental questions about life processes in all organisms (e.g. quiescence).

d) Does the Subsurface Habitat Influence Microbial Community Composition? The geologic history provides a backdrop against which we study microbial interactions with each other and the rock environment that influences them, but actively metabolizing microbes also influence their habitat (e.g., through the production of acid by-products). One of the questions discussed was whether there is an indigenous subsurface microbiota. If so, then surface-derived microbes would only supplement this subsurface community. To address this question, in light of the aforementioned questions, we need to establish whether there are similarities in microbial community composition among caves with similar habitat conditions.

Ecosystem Function

a) How Are Microbes Central to Ecosystem Function in Different Types of Karst? Based on previous cave ecosystem research, microbes, as food sources or from autotrophy, are key to the nutritional status and availability (i.e. quality and quantity of carbon substrates) and the energetic base of cave ecosystems. The base of traditional food webs drawn for most cave ecosystems has centered on a large black box that is referred to as “microorganisms”. Arrows come into and out of this box, demonstrating fluxes and processes that involve microbes. Inputs are generally considered physicochemical or nutrient constituents important for microbial pathways. For photosynthetically-based ecosystems, a critical input is light energy. Obviously, for cave ecosystems, this is not possible. Instead, inputs could be energy-rich, commonly redox-sensitive, compounds (e.g., methane, hydrogen sulfide, or other reduced substances such as iron or manganese), or allochthonous carbon compounds. Numbers are rarely added to these flux arrows, and future research needs not only to identify flux inputs and outputs to ecosystems, but to quantify flux magnitude. In general, the role of allochthonous microbes to ecosystem function (e.g., heterotrophic productivity) has rarely been studied, and only limited work has been done to characterize microbial role types within communities (e.g., autotroph or heterotroph), or to understand the scale of microbial effects and controls, on ecosystem function.

b) Are Nutrients Limiting for Microorganisms in the Subsurface? Across different karst systems and habitats, geochemical constituents vary, which should lead to differences in microbial composition and roles played in the ecosystem by microbial inhabitants. Future research investigations could fruitfully address the following questions that basically remain unanswered:

- What roles do microorganisms play in biogeochemical cycling in the subsurface? Are nitrogen, phosphorus, or carbon limiting for microorganisms in the subsurface? How do N, P, C, and S cycle in the subsurface? Are other trace elements limiting for microorganisms?
- How do heavy metals cycle in the subsurface and do microorganisms play a role in this cycling? Do microorganisms scavenge rare earth elements?
- What is role of microbes in cycling these elements from the atmosphere, to the surface, to the subsurface through the critical zone?

c) Are Microbial Community Structure and Function Linked? One of the outcomes of understanding the scales of microbial impacts to ecosystems is the characterization of niche dimensions, if microbes can be defined based on niche. Microbial niche and ecosystem dimensions could extend from the micron, to the meter, to the kilometer scale. From a conservation perspective, the stability of these dimensions requires characterization. This issue is not isolated to cave and karst microbial science research, as the categorization of microbes by niche is currently difficult to do in any field.

New developments in genomics and proteomics are providing tools with which to assess microbial function in communities and ecosystems. To investigate how community structure and function are linked we should apply these new techniques to cave and karst studies.

Disturbance

a) Are Microorganisms Agents or Victims of Disturbance? Disturbance can be natural or may originate from human influence. The degree to which microorganisms act as agents of disturbance, or are influenced by natural or anthropogenic disturbances, has received limited attention. Some excellent work has been done in Spain and France on the role of humans in causing damage to cave art through the introduction of carbon and exotic microorganisms. Some work has been done on using human indicator microbes, such as *Staphylococcus aureus*, *E. coli*, and high-temperature *Bacillus* spp. from overlying desert soils, as monitoring tools to determine human impact on caves. Beyond these few studies, however, much remains to be studied to understand the role of microorganisms in environmental issues that relate to karst. Humans are increasingly occupying karst landscapes, bringing pollution to karst aquifers and systems. The effects of this urbanization on karst microbial communities are unknown. Important future research directions and questions include:

- What factors (biological and/or geochemical) cause disturbance to karst microbial communities?
- What constitutes disturbance to a microbe in the subsurface?
- Are microbes an aspect of disturbance in karst?
- At what point does the enrichment of carbon threaten indigenous microbial communities?

In oligotrophic karst systems, even low levels of carbon enrichment may have an effect on subsurface microorganisms that are harmed by eutrophic conditions. Disasters, such as the pollution of a cave by a gasoline spill, kill many organisms, including microorganisms. Other microorganisms may be able to degrade contaminants and may move into the polluted area, replacing native bacteria killed by the contaminants. Many show caves and caves on private lands have septic tanks or sewage lines above them. When breaks or leaks occur in these systems, carbon enrichment is extreme and many organisms associated with the sewage are released into karst systems, harming the native biota. There is a wide variety of contaminants that are now polluting karst systems and we know little about most of them. The role that microorganisms play in decontaminating pollutants in the subsurface is not known either and the possibility exists that microorganisms may play a role in maintaining water quality in subsurface aquifers.

PROSPECTS FOR THE FUTURE

Justification

Microbial ecosystems are incredibly complex and difficult to study. Cave and karst microbial ecosystems provide model systems in which to conduct a range of scientific inquiries, as detailed above. Not only are caves systems intrinsically interesting and valuable, but they provide an environment with a reduced number of variables. Weathering is limited, or nearly absent, in some cave systems. In oligotrophic systems, predation by macroscopic predators can be very limited. Inputs and outputs are more easily defined. Thus, studies of complex interactions can actually be studied in an environment with a limited set of variables, making these ideal systems in which to study high-level microbial science questions.

Recommendations

Within the microbial sciences, there has been concerted effort at the national (e.g., American Society of Microbiology) and international (e.g., Society for General Microbiology, International Committee on Systematic Bacteriology) level to make overall recommendations for future research needs in the field. For cave and karst microbial sciences, we too assert that there is a fundamental need for more funding, more investigators (including individuals and consortia), and more education and communication among students within the cave and karst microbial sciences community. During breakout session discussions, three general research needs were identified:

a) Studies that take place over varying spatial and temporal scales: Varying scales, from mineral to mineral surface, from cave to cave, and from continent to continent, will address questions related to what hydrogeochemical and ecological controls may be influencing microbial community diversity and structure. Scalar investigations will also lead to a better understanding of endemism and biogeography issues. Moreover, because it unclear how microbial strains within a described species evolve over time, changes in genomes through time can profoundly affect species designations. Therefore, more studies are needed to evaluate changes in microbial community compositions and structures over time in the laboratory and in cave and karst settings.

b) Better methods and tools for understanding presence or absence of microbes: Future culture-based or genetic investigations cannot be exhaustive due to economic costs and time commitments. Therefore, more inclusive, *in situ*, and non-invasive techniques and tools to study microbial diversity and ecosystem function are needed. We expect that screening clone libraries and sequencing thousands of clones will not be feasible for large-scale projects. However, metagenomics and proteomics hold a great deal of promise for addressing these questions.

c) Good model lab for studying complex processes: A clear consensus was reached that a model cave or karst system is needed in order to address all of the microbial science questions, from an ecological perspective to the geological perspective. A model system could allow not only for basic observational and discovery-based research, but also experimentation and manipulation of the system to test disturbance issues.

Technical Barriers

Research in caves and karst is difficult to conduct. These are humid (~97-100% relative humidity) and potentially corrosive habitats. Transport of equipment needed for research studies into caves requires hauling and maneuvering over obstacles, and sometimes through narrow passages; this can easily damage sensitive instrumentation. Currently, some scientists have designed instruments to work in the cave habitat, but this has been a costly and time-consuming endeavor. Future efforts should address the need for stable, durable, economical instrumentation and equipment. Additionally, future work should also work toward standardization of scientific practices among investigative teams.

Required tools

Characterizing, monitoring, and analyzing microbial communities *in situ* will need to be addressed. Given current limitations (e.g., financial, technical, personnel, and physical), more durable and stable, yet smaller and cheaper, tools are required to perform microbial science experiments and to conduct *in situ* studies in cave and karst settings.

Instrumentation that can detect microbial metabolic activities, such as gas production or consumption (e.g., respiration), will be useful to understanding microbial ecosystem function. Essentially, a gas chromatograph interfaced with a mass spectrometer would be ideal, and should be a tool developed in the future for cave and karst research. Moreover, better, stable, quick-responding, and robust, data-logging capabilities are also needed. Currently, few caves or karst settings are logged continuously because of instrumentation sensitivity and measurement drift, which often can lead to erroneous and nonsensical interpretations.

Sampling strategies that can acquire biomass without disturbing sample integrity and community structure need to be developed. Techniques have been established for soft microbial mats, but sampling corroded rock, sediments, or solid materials will require new strategies. Maintaining the integrity of cores into punky rock is extremely difficult and requires the development of new light-weight, more effective drilling technology that can be employed in an aseptic manner.

Instrumentation and logging needs could be met with financial and technical investments in the development of autonomic microbots. Microbots could be essentially remote sensing tools "on a string" and could be used in deep karst aquifer wells or in spring fissures. Microbots could be useful to other scientific disciplines, such as hydrology, whereby 3-D mapping and characterization of habitats could be done. Microbots with geochemical sensing and sampling capabilities would be extremely useful in some karst environments. Partnerships with astrobiologists may facilitate this development.

Cave and karst science has notoriously been perceived as "cheap". Despite the fact that molecular techniques in the microbial sciences are advancing quickly, and some cave and karst researchers have already started to take advantage of the vast array of methods, including pyrosequencing and whole genome studies, most of the cave and karst microbial scientists are unfortunately lagging behind the technology cutting-edge. These researchers are limited by budgetary restrictions; molecular methods are expensive, from the standpoint of the necessary instrumentation and training, plus long-term financial commitments to keep the instruments running. Much like other molecular microbial scientists, cave and karst scientists need cheaper sequencing methods, such as retrieving >1000 bp for pyrosequencing, or faster and more reliable methods beyond 454 sequencing and we need sequencing from smaller amounts of DNA as biomass is limited in some critical study areas. Similar to other types of investigations in the Microbial Sciences, computational horsepower, with better annotation, phylogenetic, and statistical tools, are needed.

Standard Practices

The application of traditional microbiological and molecular methods may not be appropriate for cave and karst investigations. Traditional microbiological culturing methods will not work well in caves, particularly for microbes from oligotrophic habitats. However, cultivation studies are needed to study the physiology of indigenous organisms, so better cultivation methods need to be developed. Several researchers have asserted that *in situ* inoculation and incubation are critical to successfully capturing the biodiversity of organisms from caves sites. Rigorous tests of this hypothesis are necessary to determine whether culturing efforts of cave samples are growing the weeds or the indigenous microbial community. Other technical aspects that can affect the quality of the results of karst geomicrobiological studies include methods of storage and transportation of collected samples and DNA extraction and amplification. Cave samples can represent significant challenges in DNA isolation, purification, and amplification. Establishing suggested best practices for the technical aspects of geomicrobiological studies in caves would be useful to both established researchers and researchers from other fields entering into karst research.

Within the broad field of Microbial Sciences, there are obviously a number of different ways that a scientist could conduct microbiological and geomicrobiology research. There have been debates in the past regarding best practices for differentiating abiotic from biotic processes, which is a non-trivial issue. Resolving best practices in cave and karst research, while not glamorous, affects all the core studies that we wish to carry out in cave and karst geomicrobiology, and can help researchers new to the cave environment understand the differences and challenges that caves and karst offer. Further research in this area can advance our science and make important contributions to the general field of geomicrobiology. We should address best methods for preparation of samples for electron microscopy in order to accurately visualize samples without preparation artifacts or contamination. We need to encourage multidisciplinary studies that bring together geoscientists and bioscientists in research efforts to produce the best science.

Education and Outreach

The “graying” of the karst community is a significant concern. New educational initiatives and enhancement of existing cave and karst programs are needed. To attract fresh blood to the field, we need to expand and enhance educational efforts that target middle and high school students who find caves and research in caves fascinating. Some karst scientists are initiating programs to bring these students into cave and karst research studies, involving them in field and laboratory work. Science fair projects are one of the tried-and-true methods to stimulate student involvement, but summer programs are also fun and effective. We have also begun initiatives to bring public school teachers into cave and karst research so that they become part of our recruiting team. Working with existing national programs, we need to expand these efforts and improve our ability to sell karst systems as model laboratory for studying fundamental processes that occur on the surface and subsurface. Money to support such initiatives is always scarce and it’s important to expand our efforts and apply our creativity to obtain new funding.

International Cooperation

A recurring theme of the Workshop was the need to enhance the access to cave and karst information, to encourage linkages and communication among karst scientists globally, and to establish collaborative digital work spaces that lead to knowledge discovery from existing karst data. New initiatives by the University of South Florida, the University of New Mexico, the National Cave and Karst Research Institute, and the newly emerging International Cave and Karst Research Institutes Network (ICKRIN) are seeking to establish a Karst Information Portal (KIP) that will facilitate many of needed linkages and access to electronic version of various karst resources. KIP will launch in June of 2007 and efforts will intensify to add to the content and communication channels. A pilot project that is part of KIP will provide a test bed of scanning electron micrographs in an institutional repository. An accompanying collaborative work space for discussing morphological features in the images will be provided through a Drupal/Gallery installation.

Despite these promising ventures, research and pilot projects are needed to investigate:

- What methods best facilitate access to karst information, especially the grey literature and imagery?
- How do we effectively deal with intellectual property issues?
- How can we promote digital collaboration and discussion to promote knowledge discovery?

These collaborative efforts, using digital technology, will enhance knowledge discovery in cave and karst science.

Focus Group on Ecosystem Function

***Group Participants:* Kevin Simon, leader. Daniel Fong, Lara Hinderstein, Bridget Maloney, Robert Payn, Michael Vernarsky, Frank Wilhelm**

MAJOR ISSUES

The ecosystem function working group identified two major topics for future research:

What Limits Productivity in Karst?

It is now known that microbes are key mediators of energy flux in food webs of karst systems that are exclusively heterotrophic as well as those fueled by chemoautotrophy. Thus, exploration of the factors that are likely to limit microbial productivity should be of primary concern. In exclusively heterotrophic systems, dissolved organic matter (DOM) has been implicated as an important energy source for microbes and study of the influence of both the amount and composition of DOM on microbial productivity is required. The importance of other types of organic matter, such as leaves and wood, should not be ignored considering the data available to date about energy sources used by cave food animals are somewhat limited. A few data suggest inorganic nutrients (nitrogen and phosphorus) do not limit microbial productivity in heterotrophic systems, but this needs further exploration. The role of inorganic nutrients in regulating productivity in chemoautotrophic systems should be a productive avenue of research. There are some data that suggest microbial productivity may be limited because of animal grazing. This and other potential limiting factors (e.g., flooding, temperature) need to be explored. Virtually no data are available regarding energy limitation and flux in terrestrial food webs and there is a clear need for research addressing productivity in terrestrial habitats and their connectivity and relationship to aquatic habitats.

Spatial and Temporal Variation in Ecosystem Function.

Evidence is mounting that ecosystem function in karst varies spatially and temporally. The key to this variability is likely hydrology because of its role in linking subsystems within karst basins as well as linking the subsurface to other systems (e.g., surface soils and vegetation, and marine systems). Future research needs to focus on two primary issues: i) quantifying the spatial and temporal patterns in the delivery of energy and processes that transform that energy and ii) identifying the key factors regulating the spatial and temporal variation in karst. In particular, identifying the presence of “hotspots” and “hot times” for ecosystem function and the factors that lead to those situations should be addressed. Areas and times of enhanced connectivity between the surface and subsurface are likely to be important in this issue. Once the patterns and drivers of variation in ecosystem function are better understood, emphasis should shift to the consequences of spatial and temporal variability for karst ecosystems and the other ecosystems connected to karst groundwater.

JUSTIFICATION

Broad Perspective

Groundwater and karst systems in general have been considered to be organic carbon limited. In many ways, karst basins are model systems for exploring organic carbon limitation in groundwater and the role of dissolved organic carbon and microbial food webs in general for several reasons. First, the presence of caves allows unparalleled access to aquifers allowing a truly 3 dimensional approach to study surface-subsurface interaction. Second, the exclusively heterotrophic food webs and availability of systems with differing types of organic carbon input allow study of detritus in fueling food webs without the confounding effects of photoautotrophy. Indeed karst contains model systems for understanding microbial-based food webs.

In karst, the drainage network has essentially been moved from the surface to the subsurface. As a result, processes in the subsurface and ground water have replaced the ecosystem services typically provided by surface streams and rivers. Understanding subsurface processes should increase our understanding of the ecosystem services provided by karst ground water. For example, the transport of some pollutants is tied to the organic matter to which they sorb. As a result, organic matter processing in karst landscapes may play a key role in the transport and transformation of pollutants in ground water.

Karst Perspective

The ecology and evolution of animals in karst has been strongly linked to energy/productivity. Understanding what drives the productivity of karst food webs will have large implications for our understanding of the evolution of cave animals and other related issues such as patterns of biodiversity in karst. Karst ecosystems are highly susceptible to impacts from anthropogenic activities and issues such as land use and climate change will influence the spatial/temporal pattern of water flux and surface vegetation/soils in karst. Understanding and predicting the ecological consequences of such changes in karst is paramount to protect and manage these ecosystems for their continued existence. For example, understanding the hot spots and hot times of ecosystem function, and factors that drive them, should guide human activity on the surface.

APPROACHES

Comparative and descriptive research is still needed to understand food web structure and quantify the spatial and temporal patterns of energy flux in karst. However, we strongly emphasize that future studies of ecosystem function include manipulative experiments. Such manipulative experiments should begin at small spatial scales, but eventually be expanded to large scales to test hypotheses related to issues such as connectivity within subsystems in karst and between karst and other ecosystems. The use of comparative studies (e.g., gradient studies) and modeling should also be pursued. Most research conducted to date has been of limited duration and we believe a "LTER" approach for long term data collection and integration is needed.

MAJOR BARRIERS

Two major barriers to advancing ecosystem science in karst are: i) the lack of collaboration between biologists, hydrologists, geochemists and microbiologists; and ii) the lack of karst basins which can be monitored and manipulated for long periods.

There are clear transdisciplinary intersections between ecosystem ecologists and other scientists, for example hydrologists (quantifying water and solute flux through epikarst). Linking spatially explicit models of hydrologic flux to energy and nutrient flux in karst is needed. Thus, close collaboration, including joint manipulative experiments, must occur to advance the field of ecosystem science in karst. The availability of karst basins (and/or portions of basins) in which controlled, long-term manipulation and modeling can be completed will facilitate such collaboration. Ideally, these experimental basins would have good access to the subsurface (i.e., cave passage) to allow detailed sampling within karst. The ability to instrument and manipulate the surface (e.g., change surface vegetation, soils, and water flux) would be highly desirable.

The lack of basic data about ecosystem function (e.g., standing stocks and fluxes of organic matter) and the development of quantitative methods to sample the epikarst and other less accessible locations also hinders hypothesis generation and testing in karst.

TOOLS

The primary tool needed to advance ecosystem science in karst is a set of karst ecosystems that can be extensively monitored and manipulated. Ideally, the size of such systems should range from small subsystems to entire karst drainage basins. Small subbasins or caves would be sufficient to test a variety of hypotheses and generate data. Ideally, at least one karst basin would be fully instrumented (precipitation, water flux) and available for surface and subsurface manipulations. Such a location would be an ideal site to foster collaborations between ecosystem ecologists, hydrologists, geochemists and microbiologists.

Focus Group on Subterranean Biodiversity

Group Participants: David C. Culver, leader. Penelope Boston, Mary Christman, Valerie Collins, James Godwin, Horton Hobbs, Brian Holmes, John R. Holsinger, Thomas Iliffe, Jean Krejca, Jerry Lewis, Kathleen O'Conner, Tanja Pipan, Katie Schneider, Steven Taylor, and Maja Zagmajster

INTRODUCTION

Subterranean biodiversity not only encompasses the diversity of subterranean life, it also encompasses a rich diversity of research interests and research directions. The bread and butter of subterranean biodiversity studies is the description of new species, of which there is a seemingly never-ending supply. It seems to be never-ending because of the high levels of endemism of the obligate cave fauna. More intensive collections and collections from previously uncollected caves typically yield new species. Without a continuing alpha-taxonomic enterprise, further analysis and work is difficult if not impossible. This problem is neither new nor unique to subterranean biology, but it is perhaps especially acute in subterranean biology. New models for species description and species identification need to be explored. Among the suggestions that came out of the workshop were:

1. Training of "para-taxonomists" who can identify described species, and recognize undescribed species.
2. Training of taxonomists who are not group specialists but rather subterranean specialists. The emphasis here is on species description rather than high level systematics.
3. Increasing use of foreign taxonomists.

Two research themes emerged from the workshop. One was the problem of "cryptic" species that occur in different caves and cave regions, and the other was the problem of bias-free mapping of subterranean biodiversity on a regional and continental scale.

CRYPTIC SPECIES

Most cave-limited species (aquatic stygobionts and terrestrial troglobionts) have highly restricted ranges, often limited to a single cave or small group of nearby caves. No doubt the standard view of most evolutionary biologists is that cave animals invade or somehow become isolated in caves, speciate, and disperse little if at all. In fact, this is a rather uninteresting scenario, one that holds little interest to biologists who do not study caves. What it misses is that it is not true in general. Many species have relatively extensive ranges, up to hundreds of kilometers, even when cryptic species (genetically distinct but morphologically identical) are taken into account. It also misses the point about cryptic species. Why are they so numerous in caves? At a superficial level the answer is obvious. Different caves have strikingly similar environments, and adaptation should result in convergent morphology. However, the dynamics of this are not at all clear. There is often a mismatch between genetic convergence and morphological convergence.

Stygobionts and troglobionts with relatively large ranges, or at least groups of cryptic species, are often of special interest to students of evolution. It is important to note that the prime candidates for model systems for the study of evolution and development are among those species where the line between species is blurred. Examples include different populations of the Mexican cave characin, *Astyanax fasciatus*, in the Sierra de El Abra and different populations of the amphipod *Gammarus minus* in West Virginia caves. One of the most striking cases is that of cave populations of *Asellus aquaticus* in Slovenian caves. It appears that this species has invaded the same cave system (Postojna-Planina Cave System) at least twice, and probably three times!

What has not been done is the simultaneous mapping of genetic and morphological diversity of any of these "problem" species on a geographic scale. Do molecular genetic (i.e., barcoding) differences correlate with morphological differences? Are genetic and morphological variations at a site correlated? Given the frequency of cryptic species in caves, they are good model systems for the study of this phenomenon in general.

MAPPING SUBTERRANEAN BIODIVERSITY

Our capacity to store and display locational information has increased exponentially over the past decade. Several large data bases of information about stygobionts and troglobionts exist in both the U.S. and Europe, and we know at least the broad outlines of patterns of subterranean biodiversity in selected caves throughout the world. Several attempts at a quantitative synthesis have been initiated but they have been constrained by several factors:

1. Variation in intensity of sampling. Intensity and frequency of sampling in different caves varies, usually in an unknown way.
2. Sampling bias. Records of species from a cave does not necessarily mean all cave-limited species were sampled.
3. Sampling incompleteness. New species are being discovered even at well sampled caves, such as Postojna Cave in Slovenia and Vjetrenica Cave in Bosnia & Hercegovina.

There was a consensus that a broad scale geographic analysis of subterranean diversity was a very worthwhile goal, one that would yield insights into the control of subterranean diversity, assuming that sampling problems could be solved and that the appropriate environmental parameters could be measured.

The approach to the sampling problem was not to emphasize completeness, but rather to emphasize quantitative comparability. In order to accomplish this, three surrogates for total diversity were proposed:

1. Epikarst copepods collected from ceiling drips as a surrogate for total stygobiotic diversity
2. Troglobionts collected in pitfall traps as a surrogate for total troglobiotic diversity
3. Microbial community fingerprinting as a surrogate for total microbial diversity.

Quantitative sampling of epikarst copepods was pioneered by Dr. Tanja Pipan at Karst Research Institute in Postojna, Slovenia. Her technique, involving continuous filtering of drip water, is particularly appealing because it involves long term sampling (months) and because she has demonstrated the conditions for sampling completeness in terms of number of samples and length of sampling. Epikarst species diversity if high, sometimes even surpasses the rest of the stygofauna in a cave. At each drip, temperature, pH, dissolved oxygen, dissolved organic carbon, conductivity, ceiling thickness, drip type (from formation or from ceiling), ceiling thickness, and fecal coliform would be measured.

The most standard technique for sampling terrestrial cave fauna, aside from visual inspection, is the use of baited or unbaited pitfall traps. Such traps are used by almost all biologists doing general cave fauna inventories. Although to our knowledge no study of sampling completeness using pitfall traps exists, it should be relatively easy to determine sampling sufficiency using a large number of unbaited traps which are repeatedly sampled. At each pitfall trap site temperature, relative humidity, soil category, and organic carbon would be measured.

Finally, a standard 1 g of cave soil could be sampled by each pitfall trap site. They would be covered with sucrose lysis buffer to break open cells and stabilize the DNA. To assess and compare community microbial diversity, DNA will be extracted and purified to provide the basis for amplifying a 500 bp region of the genome for running on a gel using denaturing gradient gel electrophoresis (DGGE). This will provide a community fingerprint. As each band roughly corresponds to one species and the brightness of the band corresponds to numerical dominance, we can assess species richness and relative abundance across sites. Bands can be "picked" with pipette tips and sequenced to give phylogenetic information about species present.

A total of 250 caves in North America and 250 caves in Europe should provide an adequate sample. In each continent, between 10 and 12 regions would be sampled. In North America, these would include:

- Florida lime sinks
- Appalachians
- Interior Low Plateau
- Driftless Area
- Ozarks
- Black Hills
- Edwards Aquifer/Balcones Escarpment
- Guadalupe Mountains
- Scattered western areas

For each cave, location, altititude, cave length, depth, entrance size and aspect, and number of entrances would be recorded. For each region, land use, land cover, and rainfall would be recorded. These data should allow a predictive model of cave biodiversity.

The big questions are what are the patterns and what is the role of resource availability, especially organic carbon, in determining these patterns.

Understanding the Tempo and Mode of Evolution: Cave Adaptation as a Model System

Group Participants: Megan Porter, Leader. Katharina Dittmar, Ben Hutchins, William Jeffery, Tristan Lefébure, Pierre Paquin and Meredith Protas

Although cave-adapted animals have been studied for over a century, only recently have they emerged as promising model systems for understanding the tempo and mode of evolution. With advances in molecular and genomic techniques, including the increasing accessibility and affordability of full genome sequencing, it is now possible to take advantage of these model systems to understand general questions in evolutionary biology that will have interdisciplinary impacts. The unique suite of characters associated with the cave-form, termed troglomorphy, is fascinating and significant due to the convergent nature of both constructive and regressive traits across diverse taxonomic groups, which provide the opportunity to understand the specific roles of mutation, selection, development, and gene network interactions in trait evolution. Moreover, many of the traits associated with cave adaptation – increased lifespan, increased obesity, modes of locomotion, multi-modal sensory adaptation, and the loss of vision – are of critical importance from a medical perspective. The following main research themes focus on our assertion that cave species should serve as model systems to address important questions in evolutionary biology.

THE CAVE FORM

Cave adaptation is characterized by distinct morphological, physiological, and behavioral attributes (Fig. 7). The most commonly cited, and the most obvious, characters are the loss of ocular structures and pigmentation. Compared to their surface counterparts, some cave-adapted animals are physiologically characterized by lower metabolic rates, increased life spans, and changes in reproduction, while behaviorally some cave species exhibit less activity and aggression. Despite previous and ongoing investigations describing cave adaptation, few studies have looked at these physiological and behavioral characteristics comparatively across subterranean species. Recent research is finding that not all of these typical traits may be present in all cave-adapted species. On the other hand, there may be traits, such as fat deposition, that are common in cave-adaptation that have not yet been well studied. These findings illustrate that studies of the typical 'cave-form' are still required, as there may be traits that are not universal to the cave environment, or there may be crucial traits that are a result of evolution in the cave environment that have not yet been identified.

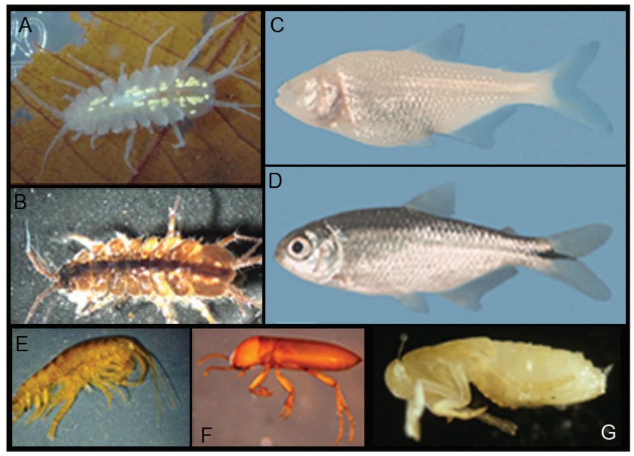

Figure 7. Examples of proposed cave-adapted model species, and where possible examples of the surface form of the same species, exhibiting troglomorphic convergence. *Asellus aquaticus* cave form (A) and surface form (B); *Astyanax mexicanus* cave form (C) and surface form (D); *Gammarus minus* (E); undescribed species of stygobiont dytiscid beetle (F); and a nymph of the planthopper *Oliarus polyphemus* (G). Photos reproduced with permission from M. Protas (A,B), W. Jeffery (C, D, G), D. Fong (E), and K. Miller (F).

MODEL SYSTEMS

Model systems are crucial to evolutionary biology studies, especially where multiple, cross-disciplinary avenues of research focus on a single species. This approach creates a community of scientists contributing knowledge on a few of the most promising systems, builds a strong informational foundation on which to conduct research, and generates momentum for funding and scientific progress. Major advances in our understanding of the development and evolution of diverse organisms and life processes have come from studies of *Drosophila melanogaster* (fruit fly), *Caenorhabditis elegans* (nematode), and *Danio rerio* (zebrafish).

In contrast to these model organisms, cave-adapted species present unique features rendering them more suitable to serve as model systems in evolutionary biology (Fig. 7). The cave-form has a suite of characters where trait polarity can be determined by comparison with surface relatives (if existing), making trait loss easy to identify.

Among the cave species currently known, there are many found across a range of phylogenetic distances (populations within one species to species spanning different phyla) that have acquired the cave-adapted traits independently (i.e. convergence of the troglomorphic form). Strikingly, and rare throughout evolution, similar cave-adapted phenotypes can be found from invertebrates to vertebrates. This characteristic allows for comparisons of the evolutionary mechanisms of particular traits across different taxonomic groups across a wide range of genetic distances. Moreover, the primary environmental cue driving the evolution of cave-adapted traits – the perpetual absence of light is obvious, and contributing environmental conditions are relatively straightforward to characterize (e.g., temperature, nutrient sources, water stress, oxygen concentration, etc.). As both subterranean terrestrial and aquatic species have cave-adapted forms, evolutionary and developmental comparisons across these habitats can help to understand what environmental parameters, in addition to the absence of light, are strong drivers of cave adaptation. For these reasons, caves contain unique organismal systems for evolutionary studies that should serve as model species but have been under utilized.

Lastly, we suggest that cave-adapted species serving as model systems must have three important characteristics: 1) the ability to be bred and raised in laboratory settings; 2) the ability to produce large numbers of fertile offspring; and 3) the existence of closely related surface form(s), either in different populations of the same species or among a group of closely related species. These criteria are important in establishing a useful laboratory organism and will help protect cave species that occur in a single location or are found in low abundances.

It would be ideal to establish model organismal systems from the main taxonomic groups found in subterranean systems, representing both terrestrial and aquatic species. However, for this to be feasible, a much greater knowledge of taxonomic relationships and ecologies, including abundance and geographic distributions, are needed. The following list represents our suggestions of species where research efforts should be focused to evaluate their potential to serve the entire evolutionary biology community as model systems. These species were chosen because a base level of knowledge already exists for evaluating their potential as model systems and upon which future studies can build. Other species may be suitable, but require much more basic ecological investigation before evaluation is possible.

Vertebrata

Characidae, Characiformes, Teleostei,

Astyanax mexicanus (De Filippi 1853)

Currently, the only subterranean model system in evolutionary biology, although relatively under utilized, is *Astyanax mexicanus*, the Mexican cave tetra. This species meets all of the listed criteria (above), and has been the focus of evolutionary and developmental studies since its discovery in the 1930s. As such, *A. mexicanus* is the best candidate for genome sequencing, which will allow for large-scale investigations of genotype-phenotype linkages leading to the observed troglomorphic forms. However, this will not be a simple undertaking, because in order for the most interesting evolutionary analyses to be performed, this requires having the unique ability to obtain sequences from a representative of both the surface and cave-adapted form. Much like the sequencing efforts for other model organisms, genome sequencing of *A. mexicanus* will necessitate a multi-institutional, and likely an international, effort.

Arthropoda

Crustacea

Asellidae, Isopoda

Asellus aquaticus (Linnaeus 1758)

Similar to *A. mexicanus*, this isopod species contains both cave-adapted and surface populations that are distributed throughout the Dinaric Karst region and attempts to rear individuals in laboratory settings are underway. While a number of phylogenetic and phylogeographic studies have been done in this group, investigations of trait evolution have not yet begun. Assuming *A. mexicanus* genome sequencing is accomplished in the near future, genome sequencing in *A. aquaticus*, of both a cave and surface form, would allow for unprecedented comparisons of trait evolution among vertebrate and arthropod models.

Gammaridae, Amphipoda

Gammarus minus (Say 1818)

In terms of trait evolution and selection, the amphipod *Gammarus minus* represents the best-studied arthropod species. Furthermore, *G. minus* can be bred in laboratory settings with minimal care. Unlike many cave species that show complete absence of eyes and pigmentation, different populations of *G. minus* exhibit varying degrees of eye and pigmentation reduction in populations that have independently colonized subterranean habitats. This range of reduction allows for the investigation of different developmental and genetic mechanisms leading to trait loss within populations of a single species.

Hexapoda

Cixiidae, Hemiptera, Insecta

Oliarus spp. (Plant hoppers)

Most described troglomorphic species occur in limestone caves; however, not all subterranean habitats are formed in limestone. Other cave types include lava tubes, which are young relative to most limestone caves, yet still contain cave-adapted faunas with reduced eyes and pigmentation. The relatively young age of lava tube habitats make troglomorphic lava tube species interesting for investigating which adaptive traits appear first. Among these taxa, the Hawaiian planthopper genus *Oliarus* consists of many related species and subspecies adapted to lava tubes, which are closely related to species from another surface dwelling genus. This species group offers a promising comparative system for studying the convergence of older, limestone occurring, lineages and much younger lava tube species.

Dytiscidae, Coleoptera, Insecta

Stygobiont diving beetles

Two subfamilies of diving beetles – Hydroporinae and Copelatinae – contain closely related species adapted to the subterranean (groundwater) and epigean realm. To date, most research of these species has concentrated on phylogenetic and taxonomic questions. Apart from the usual cave associated convergent traits, all cave forms are micropterous (e.g. exhibit reduced wings), and would represent an excellent study system for the evolution of wing reduction and loss, and its consequences for locomotion.

Eubacteria

Although there are many examples of macro-organisms adapted to cave life, only recently has attention be given to the possibility of microbial subterranean adaptation (see report by Focus Group on geomicrobiology). It is still unclear what microbial adaptations may be driven by the cave environment, or subterranean habitat in general, and whether or not these are similar to those adaptations observed in macro-organisms (e.g., decreased growth rates or metabolic rates). Identification of cave-adapted microbial species will provide model systems where potentially shorter generation times and smaller genome sizes will allow for manipulation studies and where mutation rates can be measured directly.

EVOLUTION IN KARST: MAIN RESEARCH THEMES

One of the overarching themes of evolutionary biology is to understand the tempo and mode of evolution, including selection and adaptation. Caves offer a unique system in which adaptation to a particular environment drives the evolution of specific phenotypes where both the environment and resulting traits are known with certainty. Because caves are found around the world, the cave environment is a repeated habitat where cave-adapted phenotypes occur independently in geographically isolated locations, as well as across phylogenetically diverse taxa. The following research themes illustrate how cave species can serve as exceptional model systems for answering broad evolutionary questions.

How Can We Understand the Relationship Between Convergence and Divergence?

During evolution, there is a trend towards divergence, and much of evolutionary study is devoted to understanding the resulting animal diversity. The field of ‘evo-devo’ – the study of the evolution of form – is aimed at understanding how developmental genes and patterning mechanisms result in the origins of new structures and diverse body plans. However, this focus on diversification represents only a piece of the evolutionary puzzle. Understanding how phylogenetically disparate taxa look similar is a complimentary line of research that receives much less attention. Convergence is a widespread evolutionary phenomenon. Similar modifications and reductions of ocular structures has been observed in species adapted to a number of different light-limited habitats in addition to caves, such as deep-sea dwellers, parasites, edaphobites, and burrowing animals. Yet, the interactions between convergent and divergent phenotypes and genotypes and how these interactions affect morphologies have not been comprehensively investigated. Focusing research on particularly obvious convergent morphological traits (e.g. depigmentation) and the evolution of associated homologous genes across taxonomies will help our understanding of general trait evolution, as well as the connection between sequence evolution and protein structural constraints.

Cave-adapted species are highly convergent at the morphological level, even across disparate taxonomic groups. This convergence has led to the existence of rampant cryptic genetic diversification in classically determined morphologic species (See Biodiversity section). The common occurrence of this discord between morphology and genetics in cave species offers a system that is ideal for developing methods that integrate genetic information into classical taxonomic methods. Moreover, because convergent traits can be investigated across a range of genetic distances – from morphologically dissimilar populations with similar genetic backgrounds through similar traits across different phyla – this system allows for investigations of how convergence is achieved and how the evolution of convergence and divergence are related. In particular, genomic approaches will allow biologists to investigate the relationship of convergence at multiple levels of biological organization (morphology, molecular, genomic, genome networks) and to determine how much of the genome is devoted to diversification versus convergence.

Convergent structures are most often assumed to be the result of direct selection for a particular trait. However, work in *A. mexicanus* suggests that eye loss may be the result of pleiotropy from selection for constructive traits related to feeding structures. This leads to questions about how much of the cave form is truly adaptive, how much are the adaptive structures driving the rest of the observed morphology, and are there any traits the sole product of genetic drift? This also brings into focus the problem of developmental constraint, which is a major tenant in the field of evolutionary developmental biology. Can constraints channel the evolution of cave-adapted traits? Although there has always been argument about the importance of selection versus drift as drivers of evolution (selectionist-neutralist debate), the focus now needs to shift to the relative roles of each. Cave-adapted species present an opportunity to comparatively investigate the mechanisms and evolutionary forces responsible for convergence, and to determine whether these are similar across disparate taxa.

To What Extent is the Evolution of the Cave Form Controlled by the Nature of the Karst Environment Versus Intrinsic Evolutionary Mechanisms?

Diversification, and ultimately speciation, is often the result of individuals successfully colonizing new habitats. However, what makes a particular species capable of being a successful colonizer? Pre-existing genetic variability? The evolution of novelty? Once successful colonization has occurred, what are the strongest environmental parameters driving evolution of particular traits? Are there any traits that are a phenotypic response to environmental constraints rather than the result of changes at the genetic level? How much of a role do phylogenetic constraints play in trait evolution (e.g. does your evolutionary history matter)? Are there a limited number of ways to lose traits regardless of the environmental driver, or does the environment drive the manner in which traits are lost? Karst settings offer repeated environments where these types of questions can be investigated. Obviously, the subterranean habitat exerts strong evolutionary pressures on inhabitants, as evidenced by the particular suite of traits characterized by highly cave-adapted species. Cave-adapted animals, particularly those with close surface relatives, offer the potential for genome-wide comparisons to determine how much of the cave form is genetically hard-wired versus environmentally driven. Relative to selection and adaptation, what aspects of the environment are most important? The typical cave environment is thought to be nutrient limited. Although few studies have quantitatively studied this feature, nutrient limitation may be a major factor influencing many of the typical cave-associated features, such as increased life span and reduced metabolic rates. The absence of light may also be a strong factor, as eye reductions and loss are common in a wide variety of environments that are light limited, such as deep soil and marine habitats.

What is the Timescale of Evolutionary Change?

The karst environment contains a preservation of information that is available in few surface habitats where weathering often removes the timing record of past geologic or geomorphic events. Karst, however, is partially removed from weathering processes, and therefore contains an environmental depth with respect to recording past geologic and climatic events. Essentially, karst provides windows into the evolutionary past, where time scales can be measured across generational, ecological, evolutionary, and geologic time. Studying the generational time scale is critical to understanding the evolution of the cave form, including questions such as how long are generation times in cave animals relative to surface relatives? Generation time affects the fixation of mutations, and how rapidly selection can act on advantageous alleles, leading to the convergent cave form. Ecologically, caves contain simplified ecosystems in highly stable environments where it may be possible to measure the rates of incoming invaders and the rates of population and species extinctions. When considering evolutionary timescales, caves are well constrained geologic environments containing an excellent record of time from multiple sources; dates can be determined for the age of the rock, the clastic sediments, and speleothems within a particular cave system, all providing information on the age and stability of the environment through time.

In many phylogeographic studies, ages are estimated for a particular lineage-splitting event and then these ages are compared to the timing of known geologic or climatic events *a priori* to search for possible causes. Well-studied karst systems allow for true hypothesis testing, where hypotheses about subterranean colonization, lineage diversification, speciation, and trait evolution can be generated based on the geology of the system and then tested in the evolution of the species contained within that system. Comparisons of divergence times estimated across taxa within a particular cave, karst system, region, and even across regions, will provide insight into whether or not particular climatic and geologic events have had a strong influence on colonization of the subterranean habitat. With respect to the evolution of particular traits, caves also offer the ability to put a timescale on how fast convergence and adaptation can occur. Understanding when particular species invaded the cave habitat places an upper limit on the time of trait acquisition. Furthermore, by studying species with different degrees of cave-adaptation, information can be obtained on which traits evolve more rapidly.

How Can We Use Cave Forms to Understand Mutation Rates?

An emerging theme in evolutionary biology is adaptability potential. Understanding the evolution of form requires understanding the generation of the underlying variation on which selection acts. Mutation rates are essential to understanding both the divergence times of cave species and the evolution of the cave form. Furthermore, although mutation rates are an essential component of many evolutionary theories, they are difficult to measure. Therefore, few people directly measure mutation rates in their organism of interest, and fewer still investigate broader questions such as how mutation rates change throughout the course of evolution. Cave species offer interesting systems in which it may be possible to address these questions. During the course of cave adaptation, generation times increase, affecting the ability of a species to generate genetic novelty and to respond to environmental changes. However, no one has investigated what happens to mutation rates as species adapt to the subterranean realm. As species become adapted to the karst habitat, do mutation rates slow down, speed up, or remain unchanged? Species complexes where closely related species have independently invaded multiple cave systems may provide a way to investigate these issues.

Can Cave Fauna Help Us Understand the Evolution of Behavior?

The evolution of behavior is an emerging discipline ripe for study at the molecular level. What kind and how many genes govern behavioral change? Are behavioral changes subject to conventional evolutionary mechanisms? Through what systems of the organism do the molecular changes underlying behavior act: the nervous system, the endocrine system, the reproductive system? Do behavioral changes cause evolutionary changes or *vice versa*? Despite widespread current interest in these questions, model systems are not routinely available to study the molecular basis or evolution of behavior. Cave adapted animals have the potential to make a major contribution to this field for several reasons. First, many cave animals show very strong behaviors that have evolved to maximize survival in the harsh cave environment. Classic examples are feeding, aggression, and reproductive behaviors. Second, behaviors have either appeared *de novo* in the cave environment or have been changed drastically from a related behavior that still occurs in a surface dwelling ancestor. Many ancestral behaviors also have been lost during adaptation to cave life. Finally, the existence of closely related surface and cave dwelling species, indeed sometimes divergent populations of the same species, allow powerful genetic approaches to dissect the molecular basis of evolutionary changes in cave animal behavior via hybridization. Once again, most of the research in this area has been and will continue to be conducted on *Astyanax mexicanus*, in which a suite of behavioral changes has been very well described in both the surface and cave forms. However, other cave adapted model systems offer the potential to study a wide range of behaviors and perhaps reveal unexpected convergences at the molecular level.

SUMMARY AND RECOMMENDATIONS

Recent advances in genetic tools and analyses now allow us to take advantage of the model system presented by cave faunas to address many critical biological issues in unique ways. Cave-adapted species present an unparalleled system where interactions, mechanisms, and evolutionary forces among opposing traits – constructive and regressed, convergent and divergent, selection versus drift – can be investigated. The existence of numerous, independently colonized karst systems by phylogenetically diverse species offers a comparative system par excellence. The main research themes presented here represent a highly interdisciplinary perspective, including:

- ***Taxonomy***: caves species present an ideal system for developing methods for integrating genetic diversity into classical taxonomic methods.
- ***Biodiversity***: understanding cryptic diversification and speciation in a system where both seem to be rampant.
- ***Comparative genomic studies***: genomic investigations in karst offer the potential to directly measure the proportion of the genome that is changing relative to invasion of a new habitat, convergent versus divergent features, and the role of selection versus drift in the evolution of the cave form.
- ***Ecosystem studies***: the influence of environmental parameters such as energy limitation and lack of light as driving forces of evolution can be investigated in karst.
- ***Behavior***: cave adapted animals may provide unique system to explore the evolution of behavior at the molecular

level, a major frontier in biology.

For these types of research to be successful, we require:

- The establishment of at least 2 pairs of surface and cave-adapted populations or species as model organisms.
- The funds for full genome sequencing and analysis of these model species.
- Expanded opportunities for training graduate students in aspects of the ecology of the karst habitat as well as the fields of genome science and molecular evolution.

For too long, caves have been perceived as highly specialized systems with little to offer the broader fields of biology and evolution. In reality, cave-adapted species offer unrivalled model systems in which broad biological and evolutionary questions can be investigated. The time is overripe to propel cave animals to the forefront of biological research.

Focus Group on Karst Resources and Other Applied Issues

Group Participants: Dorothy J. Vesper and Geary M. Schindel, Leaders. Barry F. Beck, John Van Brahana, Jen Cate, Debra Engler, Ralph Ewers, Joan Falkenberg, Todd Halihan, Peter Idstein, Noel Krothe, Eric Peterson, Laura Toran, George Veni, Elizabeth L. White, and Shannon Williams

MAJOR ISSUES

The major applied issues include the topics of water quality, water quantity, and process mechanisms related to geotechnical problems. The issues of greatest concern identified by the focus group are discussed below.

1. How and when contaminants are stored and transported in karst systems is not well understood. The complexity of contaminant transport is exacerbated due to the presence of trapping mechanisms, sediment transport, and episodic dilution or mobilization. The possibility for rapid injection and transport of contaminants, combined with long-term storage, results in a setting in which some contaminants may be rapidly flushed through the system whereas others may be sequestered for years or decades, with long-lasting impacts. Related questions that need to be addressed relative to contaminant transport are: 1) the movement of all phases of materials through the epikarst; 2) the exchange of water and solutes between the matrix, fracture and conduit permeability zones; and 3) the role of rapidly-changing permeability distributions (due to plugging, blowing of plugs, and evolving dissolution pathways). Closely related is the need to understand where contaminants are stored in the aquifer, particularly if they are immobile.
2. Episodic transport of sediments impacts water quality. The process, and the presence of thresholds for mobility, need to be better understood to explain differences between locations and for different events in the same location.
3. The sustainability and specific yield of karst-water resources continues to be a difficult question to address for water-use planning. Greater knowledge of and techniques for determining recharge, storage and long-term yield are needed to better manage water supplies. Tied to this question are the needs to understand climate-change impacts on water availability, the effects of drought, increased water withdrawal, and the exchange of water into and out of storage.
4. Major geotechnical issues relevant to collapse, subsidence and infiltration relate to the mechanisms of near-surface erosional processes and the associated soil mechanics. Remote sensing techniques such as geophysical monitoring can improve understanding of these relationships, but well characterized sites are needed to hone these tools.
5. Changes in land use, particularly urbanization, may affect water quality, water quantity, and collapse features. The degree to which this occurs and how it is manifested warrants additional attention.

JUSTIFICATION

The primary rationale for addressing applied issues in karst is to ensure future resource availability, be it water or land, for human and ecological populations who reside on karst. The preservation of these resources for the future requires us to address unanswered questions related to applied use.

The inconsistent and potentially-rapid throughput, coupled with the physical configuration of the subsurface, result in contaminant transport mechanisms that are not common in other aquifers. Most past applied research in karst has been site-specific; therefore, there is a need to address fundamental processes that can be readily transferred to other karst and non-karst locations.

The potential for rapid impact of karst systems also makes them a good monitoring venue for surface-activity impacts. The close connection and rapid cycling between the surface and subsurface in karst settings makes them excellent locations to study and observe the full range of interdependence of surface/subsurface interactions. The accessibility to the subsurface in karst is unique, and our ability to observe the entire system underground advances the integration of hydrology, biology, and chemistry.

Land-use planning, engineering, reliable infrastructure construction, and effective risk management requires a better understanding of the hazards associated with building on karst. Subsidence can be very slow or nearly instantaneous; end members that requires different strategies. Understanding the underlying processes and controls will help predict risks, aid in alleviating problems, and optimize our karst resources.

APPROACHES

Applied issues would be best understood if detailed, integrative (hydrology, chemistry, geophysics, geotechnical, biology) studies were conducted at well-characterized sites. The focus needs to be on fundamental processes rather than site-specific characterization and delineation. The research sites would facilitate the sharing of techniques for setting up long-term monitoring networks, finding the effective tools, and learning and testing of new equipment. A site users group would offer advice, workshops, manuals, as well as researchers available for consultation.

A “red team” approach to contamination events would allow researchers to observe rapid responses in karst systems. A karst red team, similar to those employed by volcano researchers, would be ready to mobilize quickly (within several hours) when spills occur. Not only would this approach allow team members to respond and contain early contamination-plume migration, it would also facilitate data collection and research of leading-edge contaminant behavior. Locations that offered promise for technology transfer of major processes could be pursued as long-term research sites.

MAJOR BARRIERS

Several barriers inhibit ongoing karst research in the applied field:

- Water quantity: Evaluation of storage and water budgets requires an infrastructure of both wells and springs and instrumentation to collect data on both input and output. The record must be long enough to include periods of drought, major storms, and possible threshold-exceeding impacts.
- Water quality: There are often legal and access barriers to conducting research on contaminated sites. Site specific information is often not disseminated within the scientific community due to regulatory or legal concerns by landowners. Given that contamination is an unplanned event, it rarely happens in locations that have long-term records and infrastructure for research. Availability of analytical instrumentation and support is a barrier for many researchers, particularly for analyzing organic contaminants.
- Geotechnical issues: Geotechnical knowledge and techniques are often driven by case-specific needs. Barriers include the funding and availability of different types of karst research sites in different settings that can be utilized for comparison. Being unable to predict when catastrophic failure will occur limits the study of the collapse process.

REQUIRED TOOLS

The most important tool to develop for researching applied issues is a network of research field sites that can be used for multi-disciplinary studies. Different types of data could be combined and would be available for all researchers. Teams need to incorporate practitioners and regulators as well as researchers so that information gained can be shared to groups with diverse goals and needs. The inclusion of a field site with multiple contaminants present would remove the access barriers for studying contaminant transport. An infrastructure that provides for long-term monitoring will remove the barriers related to collecting temporal-flow variation data and, eventually, include some threshold events in the dataset.

Numerous potential sites already exist: the Edwards Aquifer research site in San Antonio, Texas, the Savoy Experimental Watershed in Arkansas, Mammoth Cave National Park and adjacent lands, Bowling Green, Kentucky, and the USGS karst observatory in Leetown, West Virginia. Research is ongoing at all sites and could be expanded to a larger group of researchers via several tasks: publicizing facilities and geologic settings, obtaining a small budget for managing logistics, and formalizing access agreements.

Use of new tracer techniques and incorporation of more geochemical tools is essential. The most common tracers used in karst applied studies are fluorescent dyes that travel in the dissolved phase. More development and testing of tracers for sediment transport or different types of contaminants would greatly aid our understanding of flow. Tracers that have specific chemical characteristics (solubility, sorption potential) combined with longer-time tracer tests would allow researchers to understand contaminant transport in a more mechanistic fashion and without the need for contamination. Collaboration with researchers studying ecosystem function could help; tracking the “degradation fingerprints” of natural organic material may clarify how different types of organic compounds are cycled and transported. Isotopes are only occasionally used by practitioners in applied studies. More collaboration is needed between research geochemists and applied karst hydrogeologists.

Communication between researchers needs facilitation. While final research results are published, knowledge regarding equipment and techniques needs to be transferred as well. Establishment of equipment user groups would help this problem. This could be further solved by testing of equipment at the research field sites. Better communication between field researchers and modelers would improve both teams understanding of the flow processes and conceptual models. At the research sites the field teams could provide calibration datasets for the modelers and the modelers could provide direction on the most important data to collect.

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